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RESEARCH

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The role of endoscopic ultrasound in the diagnosis and characterization of focal liver lesions

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Abstract

Background Incidentally discovered focal liver lesions (FLLs) are a common reason for referral to hepatobiliary services. These lesions are frequently found in patients with colorectal cancer, cirrhosis, or incidentally during evaluations for abdominal pain or shock. Several established diagnostic tools such as magnetic resonance imaging (MRI), transabdominal ultrasound (US), and computed tomography (CT) are well-studied for assessing liver diseases. Endoscopic ultrasound (EUS), traditionally used for evaluating the mediastinum, biliary tract, esophagus, stomach, and pancreas, is increasingly complementing these traditional diagnostic methods in hepatology. The study aimed to delineate the endoscopic ultrasound elastography role in visualization and hepatic focal hepatic tissue differentiation of lesions in comparison to the routine radiological and laboratory methods.

Methods A cross-sectional study was conducted, we enrolled 41 patients with hepatic focal lesions, abdominal ultrasonography, triphasic CT abdomen, and Endosonography examinations were performed on all participants.

Results There was a highly significant difference between the studied groups as regards the Strain Ratio and Echogenicity (P0.01).

Conclusion Ultrasound elastography and strain ratio are promising, non-invasive, nondependent on any contrast material techniques that could significantly enhance routine grey-scale sonographic examinations of the liver by better delineating the characteristics of hepatic focal lesions.

Keywords Endoscopic Ultrasound (EUS), Elastography, Strain Ratio, Focal liver lesions (FLLs)

Introduction

Endoscopic ultrasound (EUS) has brought about a revolution in the field of medicine, particularly in endoscopy. It has been employed across numerous medical fields, including gastroenterology, cardiology, nephrology, and respiratory medicine. EUS combines endoscopy with ultrasonography, providing numerous diagnostic and therapeutic benefits. Recently, its use has expanded to include a complementary role in diagnosing certain liver diseases, along with the capability to obtain tissue biopsies [1].

The majority of focal liver lesions are detected incidentally using ultrasound (US), computed tomography (CT), or magnetic resonance imaging (MRI) during the surveillance of individuals at high risk for hepatic malignancy or during the preoperative staging of cancers. Determining the specific nature of these localized lesions is crucial, as it significantly impacts the management plan, including therapy, staging, and prognosis [2].

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Furthermore, the diagnosis of small hepatic lesions may be underestimated or missed by standard imaging investigations such as routine ultrasound. EUS has demonstrated higher diagnostic accuracy compared to US, CT, and MRI in detecting small hepatic lesions, typically those less than 1 cm. It is particularly useful in identifying suspected small hepatic metastases in patients with other primary cancers. However, only a few studies have explored the advantages of classical EUS over other imaging methods for hepatic focal lesions [3].

When compared to traditional images generated by US and computed tomography, EUS offers significant advantages, not only in diagnostic purposes but also in the capability of acquisition of tissue biopsy. The proximity of the EUS transducer to the liver and its ability to distinguish intermediate tissues and blood vessels are among the most notable benefits. EUS is an excellent technique for diagnosing and staging primary malignant tumors as well as metastatic liver disorders [4].

EUS allows for the integration of real-time elastography (RTE), which offers semi-quantitative assessments of liver parenchyma and focal lesion stiffness using color imaging. This integration enhances the ability to differentiate between malignant and benign focal liver lesions, as malignant lesions tend to be significantly stiffer. This additional tool improves EUS's capability to characterize liver masses more accurately compared to other diagnostic methods [5–7].

EUS is equipped with color, power, and pulsed Doppler capabilities, which facilitate the identification of blood vessels and the assessment of portal hypertension, collateral vessels associated with portal hypertension, and intervening vessels during procedures. Also, EUS can capture contrast-enhanced (CE) images, which assist in diagnosing localized lesions. Additionally, EUS-guided liver biopsy can be performed, which is considered safer than percutaneous biopsy, particularly in patients with liver cirrhosis and coagulation issues [5, 8].

The study seeks to explore the significance of Endoscopic Ultrasound Elastography (EUS-E) in enhancing the visualization and differentiation of focal hepatic lesions. Accurate characterization of hepatic lesions remains a critical challenge in clinical practice, as it directly impacts the diagnosis, management, and prognosis of patients with liver pathology. While traditional imaging modalities, such as ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI), along with laboratory biomarkers, are routinely employed for this purpose, they often fall short in providing sufficient specificity and sensitivity in differentiating benign from malignant lesions. EUS-E, an advanced imaging technique, has emerged as a valuable tool due to its ability to evaluate tissue stiffness—a key parameter often correlated with malignancy. By integrating elastographic analysis with the precision and proximity offered by endoscopic ultrasound, this modality has the potential to provide superior insights into the nature of hepatic lesions compared to conventional methods. The study's aim to compare EUS-E with routine radiological and laboratory approaches is particularly significant as it addresses a critical gap in current diagnostic algorithms. Establishing the role of EUS-E in focal hepatic lesion evaluation could revolutionize diagnostic workflows, reduce reliance on invasive procedures, and improve clinical decision-making in hepatobiliary disease management. Framing the research question against this background highlights its relevance to advancing both diagnostic accuracy and patient care in the field of gastroenterology and hepatology.

Therefore, the main aim of the study was to delineate the endoscopic ultrasound elastography role in visualization and hepatic focal hepatic tissue differentiation of lesions in comparison to the routine radiological and laboratory methods.

Materials and methods

This study was a cross-sectional study including patients who were presented to the internal medicine department, Hepato-gastroenterology outpatient clinics, at Kasr El Aini University Hospital and National Institute of Liver Disease during the period between January 2023 and August 2023. We recruited 41 patients who were diagnosed with hepatic focal lesion (s), and they were screened using the US, triphasic CT/MRI scan of the abdomen, and EUS. Both sexes were included. Those who have malignancies other than HCC, cardiorespiratory dysfunction that cannot tolerate the endoscopy or mental diseases were excluded from the study. The study was approved by our institution's Research Ethical Committee (MS-12–2022). Informed written consent was taken from all the participating patients or their caregivers before inclusion in the study, according to the ethical guidelines of the 1975 Declaration of Helsinki.

History taking and clinical examination were done to all the included patients including their age, gender, body mass index (BMI) by using weight in kilograms (kg) divided by the square of height in meters (m2), any comorbid diseases such as diabetes, hypertension, ischemic heart disease, valvular heart disease, family history, residency, occupation, smoking, history of previous bilharziasis or anti-bilharzial treatment, laboratory investigations such as Complete blood count (CBC), Erythrocyte sedimentation rate (ESR), C- reactive protein (CRP), Liver function assessments including serum albumin, total protein, Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline phosphatase

(ALP), Gamma-glutamyl transferase (GGT), total bilirubin, direct bilirubin, Prothrombin time (PT), Prothrombin concentration (PC) and International normalized ratio (INR), Kidney function tests including blood urea and serum creatinine, HCV anti-body (HCV-Ab) and HBV surface antigen (HBsAg) by ELISA, Serum Alpha-fetoprotein (AFP), Quantitative HCV-RNA detection using real-time polymerase chain reaction (PCR) were measured.

Abdominal Ultrasound and a Triphasic CT/MRI scan of the abdomen to confirm the presence of hepatic focal lesion (s) were done on all included patients.

Endosonography

The EUS examinations were conducted using conventional linear EUS probes (Pentax EG38-UT and EG38-70UTK, Hamburg, Germany). Initially, the lesion was classified as either benign or malignant based on standard B-mode imaging. Following this, real-time elastography was performed using a commercially available module integrated into the Hitachi EUB-8500 system (Hitachi Medical Systems Europe, Zug, Switzerland). This technology assesses tissue stiffness by measuring the degree of deformation after compression. During the EUS procedure, this compression is naturally achieved through arterial pulsations and respiratory movements. For a more in-depth understanding of the technical aspects of elastography, refer to previously published works (Giovannini et al., 2006). The region of interest was selected, and the quality of the elastographic signal was indicated on a numerical scale within the image. Tissue elasticity was overlaid onto the conventional B-mode EUS image, using color coding to indicate stiffness: blue for hard tissue, green for intermediate areas, yellow for moderately soft areas, and red for soft tissue. The elastographic and B-mode images were displayed side by side, with the full color spectrum from blue to red applied to represent the relative elasticity of the examined area. Elastographic images were interpreted in real time, and a 60-s video loop was recorded for an interobserver study.

Sample size

Sample size was calculated using STATA 14.2 software based on the following parameters, EUS elastography sensitivity in the detection of hepatic lesions 95%, specificity 100% as reported by (Okasha et al., 2020), prevalence of disease 20%, precision ± 15%, suspected dropouts 0%, and 95% confidence interval. The sample size was estimated as N = 41 patients with hepatic focal lesions. Sampling technique: Purposive sampling technique.

Statistical methods

Data management and analysis were carried out using the Statistical Package for Social Sciences (SPSS) version 28. Numerical data were presented either as means with standard deviations or as medians with ranges, depending on the data distribution. Categorical data were summarized as frequencies and percentages. Frequency estimates were based on these numbers and percentages. To assess data normality, the Kolmogorov–Smirnov and Shapiro–Wilk tests were used. The association between categorical variables was analyzed using either the Chi-square test or Fisher's exact test, depending on the data. For comparisons between two groups, the Student's t-test was used for normally distributed numerical data, while the Mann–Whitney U test was applied for non-normally distributed variables.Logistic regression was performed to evaluate the independent effect of various factors on the presence of malignant hepatic focal lesions, providing adjusted odds ratios (OR) and the magnitude of the effect of different risk factors. A 95% confidence interval (95% CI) that excludes 1.0 was considered statistically significant. The logistic regression model was built based on clinical experience, with key clinically relevant factors selected for stepwise logistic regression analysis. All statistical tests were two-tailed, and a p-value of ≤ 0.05 was considered statistically significant. The receiver operating characteristic (ROC) curve was used to determine the optimal cutoff point, sensitivity, specificity, and the area under the curve (AUC). The accuracy of the diagnostic test was evaluated based on how well it distinguished between malignant and benign cases, with the AUC providing a measure of test performance. An AUC of 1.0 indicates a perfect test, while an AUC of 0.5 indicates no diagnostic value. The traditional academic point system offers a rough guide for classifying diagnostic test accuracy:

- 0.90–1.0 = excellent (A)
- 0.80–0.90 = good (B)
- 0.70–0.80 = fair (C)
- 0.60–0.70 = poor (D)
- 0.50–0.60 = fail (F)

Results

A total of 41 patients with known hepatic focal lesion (s) were enrolled in this study. The mean age of studied patients was 51 ± 12 years, 41.5% were < 50 years, 58.5% were > 50 years, 24.4% were females and 75.6% were males. Malignant lesions were more common in older age > 50 years (79.2%).

Laboratory data showed that median of Hb level in the enrolled cases was 9.9 mg/dl with range (6.6–16), median of platelet count 180000/L with range (9000–410000), median of total leucocytic count was 4.9mcl with range (2–14.3), median of INR was 1.2 with range (1–1.8), median of ALT was 98U/L with range (9–420),

median of AST was 89U/L with range (14–380), median of Total Bilirubin was 1.1 mg/dl with range (0.1–22), median of Direct Bilirubin was 0.8 mg/dl with range (0.1–18), median of Urea level was 47 mg/dl with range (23–175), median of Creatinine level was 1.1 mg/dl with range (0.4–4.1) and median of AFP was 203 ng/ml with range (1.1–4860) (Table 1).

As expected, we found that malignant focal lesions were associated with low HB levels, elevated liver enzymes, bilirubin, alkaline phosphatase, and AFP (Table 2).

The most affected liver segment by FLLs was II 31.7%, followed by segments IV and V (14.6%), then VIII and I segment (12.2%), 46.3% had a hypoechoic lesion, 34.1% had a hyperechoic lesion, 19.5% had an isoechoic lesion, according to triphasic CT 63.4% had a malignant lesion and 36.6% had a benign lesion (Table 3) (Fig. 1).

In EUS, the median width of lesions was 3 cm, with a range of 1 to 6 cm, the median height was 2 cm, with a range of 1 to 6 cm, and the median strain ratio was 9.4, with a range of 0.2 to 49.2.

We found that most of the benign focal lesions have a low strain ratio while the malignant focal lesions have a high strain ratio. Also, malignant lesions tend to be hypoechoic (Table 4).

Strain ratio was the only significant predictor for malignant hepatic focal lesion (s). For every unit increase in strain ratio, the risk of malignancy increases nearly by three times (Table 5).

The cut-off value of the strain ratio used to diagnose the malignant lesions and differentiate these lesions from the benign lesions was 7.1, which had a sensitivity of 92.3%, specificity of 100%, PPV of 100% and NPV of 82% (Table 6) (Fig. 2).

Table 1 Laboratory data of the included patients

	Median (range)	Normal value
HB Level	9.9 (6.6–16)	Male: 14–18 g/dl, female: 12-16gm/dl
Platelet count	180,000 (9000–410000)	150,000–400000/L
The total leucocytic count	4.9 (2–14.3)	4000–11000/mcl
INR	1.2 (1–1.8)	1
ALT	98 (9–420)	4-36U/L
AST	89 (14–380)	8-33U/L
Total Bilirubin	1.1 (0.1–22)	0.1–0.2 mg/dl
Direct Bilirubin	0.8 (0.1–18)	Less than 0.3 mg/dl
Urea level	47 (23–175)	5–20 mg/dl
Creatinine level	1.1 (0.4–4.1)	0.7–1.3 mg/dl
AFP	203 (1.1–4860)	0–10 ng/ml

HB Hemoglobin, *ALT* Alanine transaminase, *AST* Aspartate Transferase, *INR* International normalised ratio, *AFP* Alpha-fetoprotein

Table 2 Laboratory findings of patients with hepatic focal lesion (s)

	Triphasic CT		
	Malignant	Benign	
	Median (range)	Median (range)	*P* value
HB Level	9.3 (6.6–12)	12 (7.4–16)	0.016
Platelet count	132,500 (9000–320000)	245,000 (31,000–410000)	0.002
TLC	4 (2–14.3)	6 (2.2–9.2)	0.056
INR	1.3 (1–1.8)	1.1 (1–1.4)	< 0.001
ALT	139 (45–420)	34 (9–88)	< 0.001
AST	150 (54–380)	36 (14–89)	< 0.001
Total Bilirubin	1.4 (0.3–22)	0.4 (0.1–1.1)	< 0.001
Direct Bilirubin	1.2 (0.3–18)	0.5 (0.1–1.1)	< 0.001
Urea level	56 (32–175)	34 (23–119)	< 0.001
Creatinine level	1.3 (0.8–4.1)	0.6 (0.4–3.5)	< 0.001
AFP	796 (3–4860)	6 (1.1–210)	< 0.001

P value < 0.05 is considered significant

HB Hemoglobin, *TLC* Total leucocytic count, *ALT* Alanine transaminase, *AST* Aspartate Transferase, *INR* International normalised ratio, *AFP* Alpha-fetoprotein

Discussion

Focal liver lesions (FLLs) pose a significant challenge during abdominal examinations as early diagnosis leads to better outcomes so, it must be taken very consciously. FLLs can be classified as benign) either solid or cystic(or malignant, and include subtypes such as hemangioma (the most common), hepatic adenoma, focal nodular hyperplasia, focal fatty change, bile duct

Table 3 Characterizations of hepatic focal lesion in the included patients

	n **= 41 (%)**
The segment site of the lesion	
I	5 (12.2)
II	13 (31.7)
III	2 (4.9)
IV	6 (14.6)
V	6 (14.6)
VI	3 (7.3)
VII	1 (2.4)
VIII	5 (12.2)
Echogenicity of the lesion	
Hypoechoic	19 (46.3)
Isoechoic	8 (19.5)
Hyperechoic	14 (34.1)
Triphasic CT	
Benign	15 (36.6)
Malignant	26 (63.4)

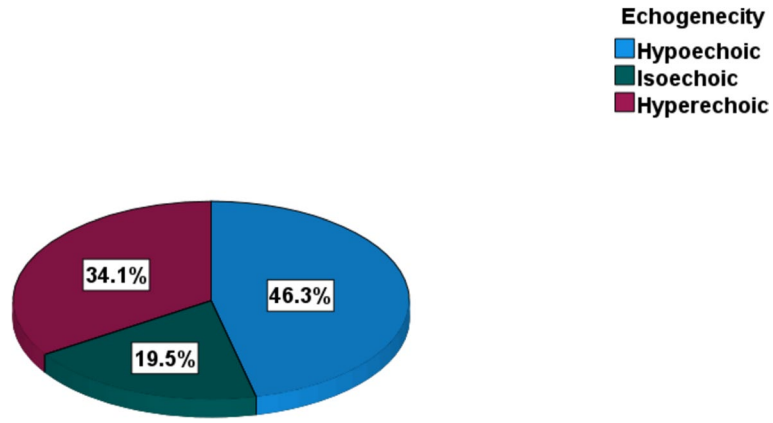

Fig. 1 Pie graph representing echogenicity of hepatic focal lesion (s) by EUS

Table 4 Comparison between malignant and benign hepatic focal lesion (s) in the included patients

	Malignant Median (range)	Benign Median (range)	*P* value
Width (Cm)	3 (1–6)	2 (1–5)	0.257
Height (Cm)	3 (1–6)	2 (1–4)	0.074
The Strain Ratio	19.2 (5.2–49.2)	1.3 (0.2–7.1)	< 0.001
Echogenecity	***n* = 26 (%)[a]**	***n* = 15 (%)[a]**	
Hypoechoic	15 (78.9)	4 (21.1)	0.036
Isoechoic	6 (75)	2 (25)	
Hyperechoic	5 (35.7)	9 (64.3)	

P value < 0.05 is considered significant

[a] Percentages were calculated within rows

Table 5 Predictor of malignant hepatic focal lesion (s)

Factors	B	S.E	*P* value	OR	95% C.I. for OR
Strain ratio	1.1	0.5	0.027	2.9	1.1–7.7
Constant	−6.2	3.1	0.042	0.002	

P-value ≤ 0.05 is considered significant

B Regression coefficients, *SE* Standard error of the coefficient, *OR Odds Ratio, 95% CI* for OR 95% confidence interval for the = Odds Ratio

Table 6 ROC curve to determine cutoff point of strain ratio that discriminate between malignant and bengin hepatic focal lesion

Variable	Cut off point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	95% CI for AUC	*P* value
SR	> 7.1	92.3	100	100	88.2	0.99	0.90–1	< 0.001

SR Strain ratio, *PPV* Positive predictive value, *NPV* Negative predictive value, *AUC* Area under the curve, *CI* Confidence interval

p value < 0.05 is considered significant

cysts, and hydatid cysts. While malignant hepatic focal lesions can be primary or secondary (metastatic). The most common primary malignant liver neoplasm is hepatocellular carcinoma (HCC), followed by cholangiocarcinoma. Other rare liver neoplasms include angiosarcomas and hepatoblastomas [9].

Endoscopic ultrasound elastography (EUS elastography) is a promising non-invasive, non-contrast imaging technique used to assess the stiffness of liver lesions. This information aids in distinguishing between benign and malignant lesions. However, lesion stiffness can also be influenced by factors such as necrosis or fibrosis. Therefore, it is essential to use EUS elastography in conjunction with other clinical information to make an accurate diagnosis [2].

The study aimed to delineate the role of endoscopic ultrasound elastography in visualization and better tissue differentiation of hepatic focal hepatic lesions in comparison to routine radiological and laboratory methods. The lesions were first classified into benign and malignant according to CT findings, and then EUS was done for comparison.

Regarding socio-demographic characteristics of the studied sample, the mean age of studied cases was 51 ± 12 years, 41.5% were < 50 years, 58.5% were > 50 years, 24.4% were females and 75.6% were males, patients with malignant masses (cancerous tumors) were significantly older (average age 61.6 years) than patients with benign lesions (non-cancerous tumors) (average age 50.4 years) (55 ± 10) ($p < 0.001$).

Concerning laboratory values for the studied sample, malignant focal lesions were associated with low HB levels, elevated liver enzymes, bilirubin, alkaline phosphatase, and AFP ($p < 0.05$).

Elastography is now incorporated into advanced ultrasound systems, with multiple studies and

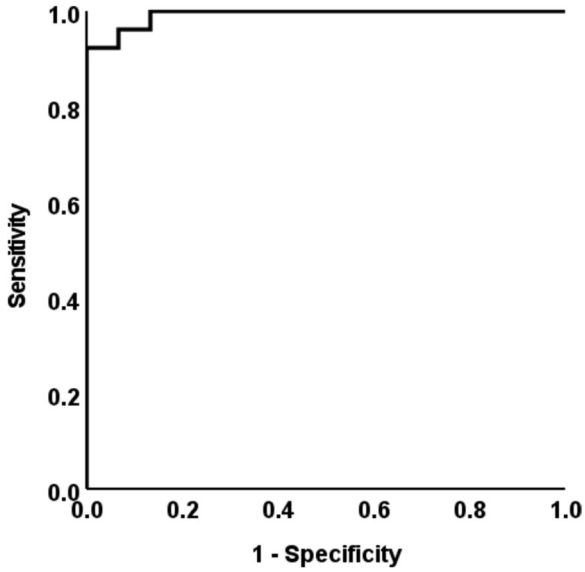

Fig. 2 ROC curve for strain ratio sensitivity in diagnosis of malignant hepatic focal lesion (s)

meta-analyses highlighting its effectiveness in identifying significant liver fibrosis and cirrhosis. Several studies reported the importance of ultrasound elastography, that is considered a technique based on tissue stiffness, it plays a crucial role in medical imaging by enabling the assessment of liver stiffness, which is essential for identifying liver fibrosis and cirrhosis stages. it could also aid in distinguishing focal liver lesions, enhancing the sensitivity and specificity of traditional grayscale ultrasound imaging [10, 11].

Once the application of EUS in the field of gastroenterology and hepatology, has increased the potentiality of diagnosis of several diseases, added on EUS elastography that has the potential to further define the tissue characteristics of benign and malignant lesions [12].

In the current study, EUS revealed that the benign focal lesions have a low strain ratio while the malignant focal lesions have a high strain ratio ($p < 0.01$).

By using the ROC curve, we found that the cut-off value of the strain ratio used to diagnose the malignant lesions and differentiate these lesions from the benign lesions was 7.1, which had a sensitivity of 92.3%, specificity of 100%, PPV of 100% and NPV of 82%, the strain ratio was the only significant predictor for the malignant hepatic focal lesion. For every unit increase in strain ratio, the risk of malignancy increases nearly by three times.

As reported in many research papers, strain elastography (SE) can be effectively used to differentiate between malignant and benign soft tissue masses which is matched with the findings in our study [13].

The main limitations of our study include that most patients in this study were confirmed by clinical and relevant imaging data, and the grade of hepatic parenchymal cirrhosis was not taken into consideration in the study. In addition, morphological characteristics of Focal Liver Lesions including size, position, boundary, shape, and color Doppler flow image pattern were not considered in our study, therefore further investigation of how to evaluate morphologically varying liver lesions and their influences on Stain ratio should be carried out.

Implications for future research

These findings pave the way for further exploration into the broader applications of EUS-E in hepatic and extrahepatic lesions. Future studies could focus on:

Validation across diverse populations

Reproducing these results in larger, multicenter studies with diverse patient populations to ensure generalizability and establish standardized protocols for clinical use.

Comparative studies

Investigating how EUS-E compares to other advanced diagnostic modalities, such as contrast-enhanced imaging techniques or molecular markers, in terms of cost-effectiveness, accessibility, and diagnostic accuracy.

Combination diagnostic models

Evaluating the integration of strain ratio findings with other imaging or laboratory biomarkers to develop comprehensive diagnostic algorithms, potentially improving outcomes in ambiguous cases.

Therapeutic implications

Exploring whether EUS-E can aid in monitoring treatment response or guiding biopsies and therapeutic interventions in liver lesions, particularly for borderline or indeterminate cases.

Implications for clinical practice

In clinical practice, the adoption of EUS-E as a diagnostic tool for hepatic focal lesions could significantly enhance patient care by:

Improved diagnostic accuracy

Providing clinicians with a non-invasive, highly accurate tool for differentiating malignant from benign hepatic lesions, thereby reducing the need for invasive diagnostic procedures, such as liver biopsies, in many cases.

Streamlined decision-making

Allowing for faster and more confident clinical decisions, particularly in scenarios where traditional imaging and laboratory results are inconclusive.

Cost-effective care

Minimizing the reliance on multiple imaging modalities or invasive diagnostics, potentially lowering healthcare costs while improving patient outcomes.

Risk stratification

Enabling more precise risk stratification of patients based on strain ratio measurements, which could guide tailored surveillance and therapeutic strategies.

The strain ratio's predictive power and ease of use make EUS-E a valuable addition to the diagnostic arsenal for hepatic lesions. As the clinical utility of this technique becomes better established, it could significantly shift the paradigm in hepatobiliary diagnostics, fostering earlier and more accurate interventions and improving overall patient outcomes.

Conclusion

Endoscopic ultrasound (EUS) has become an indispensable tool in the diagnosis and characterization of focal liver lesions (FLLs). This technique, which merges the capabilities of endoscopy with ultrasonography, offers superior imaging quality and diagnostic accuracy compared to conventional methods such as ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI).

Ultrasound elastography represents a promising addition to routine grey-scale sonographic liver examinations, offering a non-invasive and non-contrast method to enhance the characterization of hepatic lesions. It provides valuable insights into tissue stiffness, aiding in the differentiation between benign and malignant lesions. However, it is essential to acknowledge the heterogeneity within lesions, such as necrotic areas in malignant lesions or fibrotic changes in benign lesions. These variations can influence the measured stiffness on elastography, potentially causing inaccurate interpretations and necessitating cautious integration with other clinical information for accurate diagnosis and management.

Recommendations

- Further studies on a large geographical scale and on a larger sample size to emphasize our conclusion.
- Future research will further define the role of EUS elastography in clinical practice.

Abbreviations

FLLs	Focal liver lesions
MRI	Magnetic resonance imaging
US	Transabdominal ultrasound
CT	Computed tomography
EUS	Endoscopic ultrasound
RTE	Real-time elastography
CE	Contrast enhanced
HCC	Hepatocellular carcinoma
CBC	Complete blood count
ESR	Erythrocyte sedimentation rate
CRP	C- reactive protein
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ALP	Alkaline phosphatase
GGT	Gamma-glutamyl transferase
PT	Prothrombin time
PC	Prothrombin concentration
INR	International normalized ratio
HCV-Ab	HCV anti-body
HBs-Ag	HBV surface antigen
AFP	Serum Alpha fetoprotein
PCR	Real-time polymerase chain reaction

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Authors' contributions

AM did the final review & editing, GE collected the data, AA wrote the manuscript, and HH designed the methodology and helped in writing. All authors read, approved and reviewed the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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