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Original Article

Role of Ultrasound in Benign and Malignant Testicular Lesions Keeping Histopathology as Gold Standard

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ABSTRACT

Background: Testicular lesions include a heterogeneous spectrum of benign and malignant conditions, and accurate preoperative differentiation is essential to guide timely management while avoiding unnecessary invasive intervention. Grayscale ultrasound provides morphological assessment, whereas color Doppler ultrasound evaluates lesion vascularity; however, histopathology remains the definitive diagnostic standard. Objective: To evaluate the diagnostic performance of combined grayscale and color Doppler ultrasound in differentiating benign from malignant testicular lesions using histopathological examination as the gold standard. Methods: This diagnostic-accuracy cross-sectional observational study was conducted at Cancer Care Hospital & Research Center and Chughtai Lab, Lahore, over four months. Seventy-five male patients aged 18–60 years with clinically or radiologically detected testicular lesions were included. Ultrasound features including lesion type, echogenicity, margins, vascularity, vascular pattern, lesion size, and resistive index were recorded. Final ultrasound diagnosis was compared with histopathological diagnosis obtained through biopsy or orchiectomy. Sensitivity, specificity, predictive values, diagnostic accuracy, and Cohen's kappa were calculated. Results: Histopathology confirmed 45 benign and 30 malignant lesions. Ultrasound correctly identified 28 malignant and 43 benign lesions, with 2 false-positive and 2 false-negative cases. Sensitivity was 93.3%, specificity 95.6%, positive predictive value 93.3%, negative predictive value 95.6%, and diagnostic accuracy 94.7%. Cohen's kappa was 0.860, indicating excellent agreement. Hypoechogenicity, ill-defined margins, vascularity, and central or chaotic vascular patterns were strongly associated with malignancy. Conclusion: Combined grayscale and color Doppler ultrasound demonstrated excellent diagnostic performance for differentiating benign and malignant testicular lesions and may serve as an effective first-line imaging modality, particularly in resource-limited settings, while histopathology remains essential for definitive diagnosis. Keywords: Testicular lesions; grayscale ultrasound; color Doppler ultrasound; histopathology; diagnostic accuracy; benign lesions; malignant lesions; sensitivity; specificity.

INTRODUCTION

Testicular lesions comprise a heterogeneous spectrum of benign, inflammatory, traumatic, vascular, and malignant conditions, and their accurate characterization is clinically important because management differs substantially according to lesion type. Benign lesions such as cysts, inflammatory masses, and some paratesticular tumors may be treated conservatively or with limited intervention, whereas malignant intratesticular tumors require timely urological, surgical, and oncological management. Testicular germ cell tumors remain among the most clinically significant solid malignancies affecting young and middle-aged men, and early diagnosis is strongly associated with improved treatment planning, preservation of fertility-related outcomes, and reduction of disease progression. However, focal testicular lesions may show overlapping clinical and imaging appearances, making reliable

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differentiation between benign and malignant pathology essential before definitive treatment decisions are made (1).

Clinical examination alone is often insufficient for distinguishing benign from malignant testicular lesions because both groups may present with scrotal swelling, pain, palpable abnormality, or incidental detection during imaging. In addition, some inflammatory or benign vascular lesions can mimic malignancy, while small malignant lesions may be clinically subtle or non-palpable. This diagnostic uncertainty creates a practical clinical problem: delayed recognition of malignancy may postpone curative treatment, whereas overdiagnosis of benign disease may contribute to unnecessary orchiectomy or avoidable invasive intervention. Therefore, a non-invasive, accessible, and diagnostically reliable imaging approach is needed to support preoperative risk stratification and guide appropriate referral, biopsy, surgery, or conservative management (2).

Ultrasonography is widely accepted as the first-line imaging modality for assessment of scrotal and testicular abnormalities because it is safe, non-invasive, inexpensive, radiation-free, and readily available in routine clinical practice. Grayscale ultrasound provides detailed morphological evaluation of lesion size, echogenicity, internal composition, borders, and relationship with surrounding testicular parenchyma, whereas color Doppler ultrasound adds functional information by assessing vascularity and vascular distribution. Malignant lesions are more commonly described as solid, hypoechoic, ill-defined, and internally vascular, while benign lesions are more likely to demonstrate cystic morphology, smooth margins, absent vascularity, or peripheral vascularity depending on the underlying pathology. The combined interpretation of grayscale and Doppler features may therefore improve diagnostic discrimination compared with either modality alone (3).

Despite its central clinical role, conventional ultrasound has important limitations. Imaging features of benign and malignant testicular lesions may overlap, particularly in inflammatory lesions, infarction, hematoma, small tumors, and lesions with atypical vascular patterns. Advanced modalities such as contrast-enhanced ultrasound, elastography, and magnetic resonance imaging may improve lesion characterization in selected cases, but these techniques are not universally available, may increase cost, and may not be feasible in resource-limited healthcare settings. Histopathological examination remains the definitive reference standard because it confirms lesion nature, establishes tumor subtype, and provides prognostic information required for further management. However, histopathology is invasive and is commonly obtained after biopsy, surgical exploration, or orchiectomy; therefore, accurate preoperative ultrasound assessment remains clinically valuable for guiding decision-making before tissue confirmation (4).

Previous studies have reported useful diagnostic performance of ultrasound and Doppler-based assessment in testicular pathology, but reported sensitivity, specificity, and accuracy vary according to study population, lesion spectrum, operator expertise, imaging criteria, and availability of histopathological confirmation. This variability indicates that diagnostic performance cannot be assumed to be uniform across clinical settings. In Pakistan and similar resource-limited contexts, grayscale and color Doppler ultrasound remain the most accessible diagnostic tools for testicular lesion evaluation, yet local evidence directly comparing combined ultrasound findings with histopathology remains limited. This creates a knowledge gap regarding how accurately routinely available ultrasound can differentiate benign from malignant testicular lesions in the local clinical population and whether specific grayscale and Doppler features can support malignancy risk stratification before definitive histological diagnosis (5).

Using a PICO-based framework, the population of interest in this study was adult male patients with clinically or radiologically detected testicular lesions; the index test was combined grayscale and color Doppler ultrasound; the comparator and reference standard was histopathological diagnosis obtained through biopsy or orchiectomy; and the outcomes were sensitivity, specificity, positive predictive value, negative predictive value, overall diagnostic accuracy, and agreement between ultrasound and histopathology. Therefore, the objective of this study was to evaluate the diagnostic performance of combined grayscale and color Doppler ultrasound in differentiating benign from malignant testicular lesions using histopathological examination as the gold standard.

MATERIALS AND METHODS

This diagnostic-accuracy cross-sectional observational study was conducted to evaluate the performance of combined grayscale and color Doppler ultrasound for differentiating benign from malignant testicular lesions, using histopathological examination as the reference standard. The study design was selected because both the index test and reference standard were assessed within the same patient population, allowing direct estimation of sensitivity, specificity, predictive values, overall diagnostic accuracy, and agreement between ultrasound diagnosis and final histopathological diagnosis. The study was planned according to standardized principles for reporting diagnostic-accuracy research, with predefined eligibility criteria, operational definitions of imaging variables, and comparison of the index test against a histopathological gold standard (6).

The study was carried out at Cancer Care Hospital & Research Center and Chughtai Lab, Lahore, Pakistan, over a four-month period after formal synopsis approval. The source population comprised male patients presenting with clinically or radiologically detected testicular lesions who subsequently underwent histopathological confirmation through biopsy or orchiectomy. Eligible participants were adult male patients aged 18 to 60 years with a testicular lesion detected on clinical examination or prior radiological assessment and for whom both grayscale/color Doppler ultrasound and histopathological diagnosis were available. Patients were excluded if they had a previous history of testicular surgery or scrotal intervention, incomplete ultrasound or histopathological records, or technical limitations that prevented adequate sonographic assessment of the lesion. Participants were selected through non-probability convenience sampling from patients fulfilling the eligibility criteria during the study period.

Patients were recruited after assessment of eligibility, and written informed consent was obtained before inclusion. Clinical and demographic information was collected using a structured data collection proforma. Recorded variables included age, duration of symptoms, presenting complaint, relevant clinical history, lesion location, lesion size, grayscale ultrasound features, Doppler ultrasound features, method of histological confirmation, and final histopathological diagnosis. Presenting complaints were categorized as pain, swelling, swelling with pain, or incidental detection. Relevant history was categorized as trauma, infection, family history, or no significant history. Lesion location was recorded as right-sided, left-sided, or bilateral. Histological confirmation method was recorded as biopsy or orchiectomy.

All included patients underwent grayscale and color Doppler ultrasound examination of the scrotum and testes using a standardized scanning approach. Both testes were examined in longitudinal and transverse planes, and the abnormal lesion was assessed systematically. Grayscale ultrasound was used to evaluate lesion type, echogenicity, margins, size, and associated findings. Lesion type was operationally defined as solid, cystic, or mixed according to internal composition. Echogenicity was classified as hypoechoic, hyperechoic, or isoechoic relative to adjacent testicular parenchyma. Lesion margins were classified as well-defined when the lesion showed a clear interface with surrounding tissue and ill-defined when the boundary was indistinct or infiltrative. Lesion size was measured in millimeters. Associated findings, including hydrocele, hematocele, atrophy, or absence of associated abnormality, were recorded where present.

Color Doppler ultrasound was performed to evaluate vascularity and vascular distribution within or around the lesion. Vascularity was recorded as present when Doppler flow was detected and absent when no internal or peripheral flow was identified. Vascular pattern was categorized as absent, peripheral, central, or chaotic. Peripheral vascularity referred to flow predominantly around the lesion margin, central vascularity referred to flow within the lesion, and chaotic vascularity referred to irregular or disorganized intralesional vascular distribution. Resistive index was recorded when a measurable Doppler waveform was obtained. The final ultrasound diagnosis was classified as benign or malignant using combined grayscale and Doppler features. Lesions with cystic morphology, well-defined margins, absent or predominantly peripheral vascularity, and benign inflammatory features were classified as benign, whereas lesions with solid or mixed morphology, hypoechogenicity, ill-defined margins, and central or chaotic vascularity were classified as suspicious for malignancy.

Histopathological diagnosis obtained through biopsy or orchiectomy was used as the reference standard. Histopathological findings were categorized as benign or malignant for diagnostic-accuracy analysis. Benign histological categories included adenomatoid tumor, epididymitis, cyst, and orchitis, while malignant histological categories included non-seminomatous germ cell tumor, seminoma, and lymphoma. The ultrasound diagnosis was compared with the histopathological diagnosis to construct a 2×2 diagnostic table. True positive cases were defined as lesions diagnosed as malignant on ultrasound and confirmed as malignant on histopathology. True negative cases were lesions diagnosed as benign on ultrasound and confirmed as benign on histopathology. False positive cases were lesions diagnosed as malignant on ultrasound but benign on histopathology, and false negative cases were lesions diagnosed as benign on ultrasound but malignant on histopathology.

Several steps were applied to reduce bias and improve internal validity. Eligibility criteria were defined before data analysis to minimize inappropriate case inclusion. Only patients with both ultrasound assessment and histopathological confirmation were included, reducing uncertainty in outcome classification. Ultrasound variables were recorded using predefined categories to minimize measurement variability and improve reproducibility. The same reference standard, histopathological diagnosis, was used for final classification of benign and malignant lesions. Data were checked for completeness before analysis, and cases with incomplete imaging or histopathological information were excluded. Potential confounding by demographic and lesion-related characteristics was addressed by comparing clinical, grayscale, and Doppler features between benign and malignant groups and by interpreting diagnostic performance in relation to histopathological status rather than clinical suspicion alone.

The sample size was calculated using an expected sensitivity of 88.7%, disease prevalence of 51.4%, 95% confidence level, and 10% absolute precision. The required sample size was approximately 75 patients, and this number was used as the final study sample. The primary outcome measures were sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy of combined grayscale and color Doppler ultrasound for differentiating benign from malignant testicular lesions. The secondary outcome measures included agreement between ultrasound and histopathology using Cohen's kappa and associations between individual ultrasound features and malignant histopathology.

Data were analyzed using SPSS. Continuous variables were summarized as mean and standard deviation when normally distributed and as median with interquartile range when distributional assumptions were not met. Categorical variables were summarized as frequencies and percentages. The normality of continuous variables was assessed before group comparison. Independent-samples t-test was used for normally distributed continuous variables, while the Mann–Whitney U test was used for non-normally distributed variables. Associations between categorical ultrasound features and histopathological diagnosis were assessed using the chi-square test or Fisher's exact test where expected cell counts were small. Diagnostic performance was calculated from the 2×2 table using standard formulas for sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy. Cohen's kappa was used to assess agreement between ultrasound diagnosis and histopathological diagnosis. A p-value of less than 0.05 was considered statistically significant. Complete-case analysis was used, and no statistical imputation was applied.

Ethical standards were maintained throughout the study in accordance with accepted principles for research involving human participants (7). Written informed consent was obtained from all participants before inclusion. Confidentiality was maintained by anonymizing patient data and restricting access to study records. Data integrity was ensured through use of a structured proforma, predefined variable categories, verification of entries before analysis, and consistent coding of clinical, ultrasound, Doppler, and histopathological variables. The diagnostic criteria, outcome definitions, and statistical procedures were specified to allow reproducibility by other researchers evaluating similar patient populations.

RESULTS

A total of 75 male patients with testicular lesions were included in the study, all of whom underwent grayscale and color Doppler ultrasound followed by histopathological confirmation. As shown in Table 1, the mean age of the participants was 41.48 ± 16.50 years, indicating a broad adult age distribution within the eligible age range. The mean duration of symptoms was 73.80 ± 103.59 days, showing considerable variability in clinical presentation and suggesting that some patients presented late after symptom onset. The mean lesion size was 27.78 ± 12.38 mm, while the mean resistive index was 0.65 ± 0.14 among recorded Doppler measurements.

Table 2 summarizes the clinical background and presenting symptoms of the study population. Most patients had no significant relevant history, accounting for 46 of 75 cases (61.3%). Among those with a relevant history, trauma was reported in 12 patients (16.0%), family history in 11 patients (14.7%), and infection in 6 patients (8.0%). Swelling was the most frequent presenting complaint, observed in 31 patients (41.3%), followed by pain in 24 patients (32.0%).

Combined swelling and pain were reported in 16 patients (21.3%), whereas 4 lesions (5.3%) were detected incidentally. These findings indicate that most lesions were clinically symptomatic, with swelling alone or pain alone accounting for nearly three quarters of all presentations.

The grayscale ultrasound findings are presented in Table 3. Lesions were slightly more common on the left side, with 35 cases (46.7%), compared with 32 right-sided lesions (42.7%) and 8 bilateral lesions (10.7%). In terms of lesion composition, solid lesions were the predominant type, observed in 47 patients (62.7%), followed by mixed lesions in 18 patients (24.0%) and cystic lesions in 10 patients (13.3%). Hypoechoic echogenicity was the most common sonographic pattern, seen in 43 lesions (57.3%), while hyperechoic and isoechoic patterns were each found in 16 lesions (21.3%). Ill-defined margins were observed in 52 lesions (69.3%), compared with well-defined margins in 23 lesions (30.7%). Overall, the grayscale profile of the cohort showed a predominance of solid, hypoechoic, and ill-defined lesions, which are features commonly considered suspicious in testicular lesion assessment.

Color Doppler ultrasound findings and associated abnormalities are shown in Table 4. Doppler vascularity was detected in 53 lesions (70.7%), whereas 22 lesions (29.3%) had no demonstrable vascularity. Regarding vascular pattern, peripheral vascularity was the most frequent pattern, present in 24 cases (32.0%), followed by absent vascular pattern in 21 cases (28.0%), chaotic vascularity in 18 cases (24.0%), and central vascularity in 12 cases (16.0%).

Associated findings were absent in 36 patients (48.0%). Among associated abnormalities, hydrocele was the most common, identified in 23 patients (30.7%), followed by hematocele in 11 patients (14.7%) and atrophy in 5 patients (6.7%). These findings show that Doppler vascularity was present in more than two thirds of lesions, while central or chaotic vascularity together accounted for 30 of 75 cases (40.0%).

Histopathological findings are detailed in Table 5. Final histopathology confirmed benign lesions in 45 patients (60.0%) and malignant lesions in 30 patients (40.0%). Among benign lesions, adenomatoid tumor was the most frequent diagnosis, reported in 13 cases (17.3% of the total sample), followed by epididymitis in 12 cases (16.0%), cyst in 10 cases (13.3%), and orchitis in 10 cases (13.3%). Among malignant lesions, non-seminomatous germ cell tumor was the most common diagnosis, observed in 13 patients (17.3%), followed by seminoma in 10 patients (13.3%) and lymphoma in 7 patients (9.3%). Histological confirmation was obtained through orchiectomy in 56 patients (74.7%) and biopsy in 19 patients (25.3%), indicating that most diagnoses were confirmed through surgical tissue specimens.

Table 6 demonstrates the association between selected ultrasound features and malignant histopathology. Hypoechoic echogenicity was present in 43 lesions, of which 30 were malignant, giving a malignancy proportion of 69.8% among hypoechoic lesions. In contrast, all 32 non-hypoechoic lesions were benign. This association was statistically significant, with Fisher's exact $p < 0.001$ and an estimated odds ratio of 146.85 using continuity correction. Ill-defined margins were also significantly associated with malignancy. Of 52 lesions with ill-defined margins, 30 were malignant, giving a malignancy proportion of 57.7%, whereas all 23 well-defined lesions were benign.

The association between ill-defined margins and malignancy was statistically significant, with $p < 0.001$ and an estimated odds ratio of 63.71. Doppler vascularity was present in 53 lesions, including all 30 malignant lesions and 23 benign lesions, while all 22 avascular lesions were benign. This yielded a significant association between vascularity and malignancy, with $p < 0.001$ and an estimated odds ratio of 58.40. The strongest discriminatory feature was central or chaotic vascularity. All 30 lesions with central or chaotic vascularity were malignant, whereas all 45 lesions with absent or peripheral vascularity were benign, producing a malignancy proportion of 100.0% in the central/chaotic vascularity group and 0.0% in the absent/peripheral vascularity group. This association was highly significant, with $p < 0.001$ and the largest estimated odds ratio among the evaluated sonographic features.

The diagnostic cross-tabulation of combined grayscale and color Doppler ultrasound against histopathology is presented in Table 7. Ultrasound classified 30 lesions as malignant and 45 lesions as benign. Among the 30 histopathologically malignant lesions, ultrasound correctly identified 28 as malignant, while 2 malignant lesions were incorrectly classified as benign, representing false-negative results.

Among the 45 histopathologically benign lesions, ultrasound correctly classified 43 as benign, while 2 benign lesions were incorrectly classified as malignant, representing false-positive results. Therefore, the ultrasound diagnosis agreed with histopathology in 71 of 75 cases and disagreed in 4 cases.

Table 8 shows the inferential agreement analysis between ultrasound diagnosis and histopathological diagnosis. The association between combined ultrasound diagnosis and histopathological diagnosis was statistically significant, with a Pearson chi-square value of 55.557 and $p < 0.001$. Cohen's kappa was 0.860, indicating excellent agreement between ultrasound-based classification and histopathological diagnosis. This high kappa value shows that the observed agreement was substantially greater than expected by chance alone.

The diagnostic-performance estimates are summarized in Table 9. Combined grayscale and color Doppler ultrasound achieved a sensitivity of 93.3% (28/30; 95% CI: 78.7–98.2%), indicating that the modality correctly identified most malignant lesions. Specificity was 95.6% (43/45; 95% CI: 85.2–98.8%), showing that ultrasound correctly excluded malignancy in most benign lesions.

The positive predictive value was 93.3% (28/30; 95% CI: 78.7–98.2%), meaning that most lesions classified as malignant on ultrasound were confirmed malignant on histopathology. The negative predictive value was 95.6% (43/45; 95% CI: 85.2–98.8%), indicating a high probability that lesions classified as benign on ultrasound were truly benign. Overall diagnostic accuracy was 94.7% (71/75; 95% CI: 87.1–97.9%), reflecting excellent overall agreement between combined ultrasound assessment and histopathological diagnosis.

Taken together, the results show that combined grayscale and color Doppler ultrasound demonstrated high diagnostic performance for differentiating benign from malignant testicular lesions in this cohort. The most informative ultrasound features associated with malignancy were central or chaotic vascularity, hypoechoic echogenicity, ill-defined margins, and Doppler vascularity. Among these, central or chaotic vascularity showed the strongest discriminatory value, as all lesions with this pattern were histopathologically malignant. The high sensitivity, specificity, predictive values, and diagnostic accuracy support the clinical value of combined ultrasound assessment as a first-line diagnostic tool, while the presence of false-positive and false-negative cases confirms that histopathology remains essential for definitive diagnosis.

Table 1. Baseline clinical and continuous characteristics of the study population

Variable	N	Mean ± SD
Age, years	75	41.48 ± 16.50
Duration of symptoms, days	75	73.80 ± 103.59
Lesion size, mm	75	27.78 ± 12.38
Resistive index	75	0.65 ± 0.14

Most patients had no significant relevant clinical history, reported in 46 cases (61.3%). Trauma was reported in 12 patients (16.0%), family history in 11 patients (14.7%), and infection in 6 patients (8.0%). Swelling was the most common presenting symptom, observed in 31 patients (41.3%), followed by pain in 24 patients (32.0%), combined swelling and pain in 16 patients (21.3%), and incidental detection in 4 patients (5.3%).

Table 2. Clinical history and presenting symptoms

Variable	Category	Frequency, n	Percentage, %
Relevant history	None	46	61.3
	Trauma	12	16.0
	Family history	11	14.7
	Infection	6	8.0
Presenting symptoms	Swelling	31	41.3
	Pain	24	32.0
	Swelling and pain	16	21.3
	Incidental finding	4	5.3

Left-sided lesions were slightly more frequent than right-sided lesions. Lesions were located on the left side in 35 patients (46.7%), on the right side in 32 patients (42.7%), and bilaterally in 8 patients (10.7%). On grayscale ultrasound, solid lesions were the most common lesion type, accounting for 47 cases (62.7%), followed by mixed lesions in 18 cases (24.0%) and cystic lesions in 10 cases (13.3%). Hypoechoic echogenicity was the most frequent echogenic pattern, observed in 43 lesions (57.3%), while hyperechoic and isoechoic appearances were each observed in 16 lesions (21.3%). Ill-defined margins were present in 52 lesions (69.3%), whereas 23 lesions (30.7%) had well-defined margins.

Table 3. Grayscale ultrasound findings

Variable	Category	Frequency, n	Percentage, %
Lesion location	Left	35	46.7
	Right	32	42.7
	Bilateral	8	10.7
Lesion type	Solid	47	62.7
	Mixed	18	24.0
	Cystic	10	13.3
Echogenicity	Hypoechoic	43	57.3
	Hyperechoic	16	21.3
	Isoechoic	16	21.3
Margins	Ill-defined	52	69.3
	Well-defined	23	30.7

On color Doppler ultrasound, vascularity was present in 53 lesions (70.7%) and absent in 22 lesions (29.3%). Peripheral vascularity was the most common vascular pattern, observed in 24 cases (32.0%), followed by absent vascular pattern in 21 cases (28.0%), chaotic vascularity in 18 cases (24.0%), and central vascularity in 12 cases (16.0%). Among associated findings, hydrocele was observed in 23 patients

(30.7%), hematocele in 11 patients (14.7%), and testicular atrophy in 5 patients (6.7%), while 36 patients (48.0%) had no associated finding.

Table 4. Color Doppler ultrasound and associated findings

Variable	Category	Frequency, n	Percentage, %
Vascularity	Present	53	70.7
	Absent	22	29.3
Vascular pattern	Peripheral	24	32.0
	Absent	21	28.0
	Chaotic	18	24.0
	Central	12	16.0
Associated findings	None	36	48.0
	Hydrocele	23	30.7
	Hematocele	11	14.7
	Atrophy	5	6.7

Histopathology confirmed benign lesions in 45 patients (60.0%) and malignant lesions in 30 patients (40.0%). Among benign diagnoses, adenomatoid tumor was identified in 13 cases (17.3%), epididymitis in 12 cases (16.0%), cyst in 10 cases (13.3%), and orchitis in 10 cases (13.3%). Among malignant diagnoses, non-seminomatous germ cell tumor was the most common malignancy, observed in 13 cases (17.3%), followed by seminoma in 10 cases (13.3%) and lymphoma in 7 cases (9.3%). Histological confirmation was obtained through orchiectomy in 56 patients (74.7%) and biopsy in 19 patients (25.3%).

Table 5. Histopathological diagnosis and method of confirmation

Variable	Category	Frequency, n	Percentage, %
Final histopathological diagnosis	Benign	45	60.0
	Malignant	30	40.0
Benign histology	Adenomatoid tumor	13	17.3
	Epididymitis	12	16.0
	Cyst	10	13.3
	Orchitis	10	13.3
Malignant histology	Non-seminomatous germ cell tumor	13	17.3
	Seminoma	10	13.3
	Lymphoma	7	9.3
Confirmation method	Orchiectomy	56	74.7
	Biopsy	19	25.3

Several grayscale and Doppler features showed strong associations with malignant histopathology. Hypoechoic echogenicity was present in 43 lesions, of which 30 were malignant and 13 were benign, while all 32 non-hypoechoic lesions were benign. Ill-defined margins were observed in 52 lesions, including all 30 malignant lesions and 22 benign lesions, whereas all 23 well-defined lesions were benign. Doppler vascularity was present in 53 lesions, including all 30 malignant lesions and 23 benign lesions, while all 22 avascular lesions were benign. The strongest discriminatory feature was central or chaotic vascularity: all 30 lesions with central or chaotic vascularity were malignant, whereas all 45 lesions with absent or peripheral vascularity were benign.

Table 6. Association of selected ultrasound features with malignant histopathology

Ultrasound feature	Malignant, n/N (%)	Benign, n/N (%)	Statistical test	Odds ratio*	95% CI for odds ratio*	p-value
Hypoechoic echogenicity vs non-hypoechoic echogenicity	30/43 (69.8)	13/43 (30.2)	Fisher's exact test	146.85	8.36–2578.77	<0.001
Ill-defined margins vs well-defined margins	30/52 (57.7)	22/52 (42.3)	Fisher's exact test	63.71	3.67–1105.46	<0.001
Doppler vascularity present vs absent	30/53 (56.6)	23/53 (43.4)	Fisher's exact test	58.40	3.37–1013.38	<0.001
Central/chaotic vascularity vs absent/peripheral vascularity	30/30 (100.0)	0/30 (0.0)	Fisher's exact test	5551.00	107.23–287355.94	<0.001

*Odds ratios and 95% confidence intervals were calculated using continuity correction because one or more cells contained zero values.

Combined grayscale and color Doppler ultrasound classified 30 lesions as malignant and 45 lesions as benign. Compared with histopathology, ultrasound correctly identified 28 of 30 malignant lesions and 43 of 45 benign lesions. There were 2 false-positive and 2 false-negative cases. The association between ultrasound diagnosis and histopathological diagnosis was statistically significant.

Table 7. Cross-tabulation of combined ultrasound diagnosis against histopathology

Ultrasound diagnosis	Histopathology malignant, n	Histopathology benign, n	Total, n
Malignant	28	2	30
Benign	2	43	45
Total	30	45	75

Table 8. Association between ultrasound diagnosis and histopathological diagnosis

Analysis	Value	95% CI	p-value
Pearson chi-square	55.557	—	<0.001
Cohen's kappa	0.860	—	<0.001

The diagnostic performance of combined grayscale and color Doppler ultrasound was high across all indices. Sensitivity was 93.3%, specificity was 95.6%, positive predictive value was 93.3%, negative predictive value was 95.6%, and overall diagnostic accuracy was 94.7%.

Table 9. Diagnostic performance of combined grayscale and color Doppler ultrasound

Diagnostic parameter	Numerator/denominator	Value, %	95% CI, %
Sensitivity	28/30	93.3	78.7–98.2
Specificity	43/45	95.6	85.2–98.8
Positive predictive value	28/30	93.3	78.7–98.2
Negative predictive value	43/45	95.6	85.2–98.8
Diagnostic accuracy	71/75	94.7	87.1–97.9

Overall, combined grayscale and color Doppler ultrasound showed excellent diagnostic performance for differentiating benign from malignant testicular lesions when compared with histopathology. Hypoechoic echogenicity, ill-defined margins, Doppler vascularity, and central or chaotic vascularity were significantly associated with malignant histopathology, with central or chaotic vascularity demonstrating the strongest discriminatory value in this cohort.

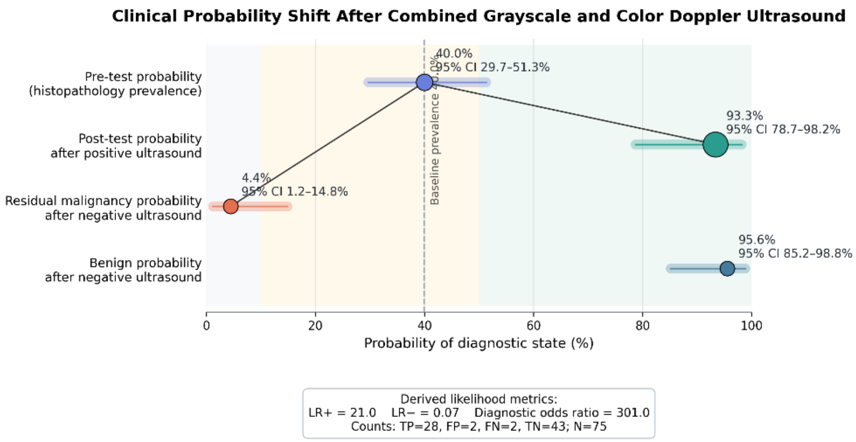

Figure 1 Clinical Probability Shift After Combined Grayscale And Color Doppler Ultrasound

The baseline histopathological malignancy prevalence was 40.0% (30/75; 95% CI: 29.7–51.3%), but a positive combined grayscale and color Doppler ultrasound result increased the post-test probability of malignancy to 93.3% (28/30; 95% CI: 78.7–98.2%), corresponding to a strong positive likelihood ratio of 21.0. Conversely, a negative ultrasound result reduced the residual probability of malignancy to 4.4% (2/45; 95% CI: 1.2–14.8%) and increased the probability of benign pathology to 95.6% (43/45; 95% CI: 85.2–98.8%), with a low negative likelihood ratio of 0.07. The diagnostic odds ratio of 301.0 indicates marked separation between malignant and benign disease states, supporting the clinical value of combined ultrasound as a high-yield first-line stratification tool while still confirming the need for histopathological verification in discordant cases.

DISCUSSION

This study evaluated the diagnostic performance of combined grayscale and color Doppler ultrasound in differentiating benign from malignant testicular lesions using histopathological examination as the reference standard. The findings demonstrated high diagnostic performance, with ultrasound correctly classifying 71 of 75 lesions and achieving a sensitivity of 93.3%, specificity of 95.6%, positive predictive value of 93.3%, negative predictive value of 95.6%, and overall diagnostic accuracy of 94.7%. The strong agreement between ultrasound diagnosis and histopathology, reflected by a Cohen's kappa value of 0.860, indicates that combined morphological and vascular assessment provides clinically meaningful discrimination between benign and malignant testicular pathology. These findings support the role of conventional ultrasound as a highly useful first-line imaging modality in patients with testicular lesions, particularly because scrotal ultrasound is widely available, non-invasive, cost-effective, and capable of real-time assessment of both lesion morphology and vascular behavior (8).

The high sensitivity observed in this study is clinically important because false-negative imaging assessment of a malignant testicular lesion may delay definitive treatment and increase the risk of progression. In the present cohort, ultrasound missed only 2 of 30 malignant lesions, corresponding to a false-negative proportion of 6.7% among histopathologically malignant cases. Conversely, the high specificity indicates that ultrasound correctly identified 43 of 45 benign lesions, with only 2 false-positive cases. This finding has direct clinical relevance because benign testicular and paratesticular lesions may sometimes be managed conservatively or with limited intervention, whereas overclassification as malignant may lead to avoidable anxiety, unnecessary invasive procedures, or radical orchiectomy. Therefore, the combination of high sensitivity and high specificity suggests that grayscale and color Doppler ultrasound can substantially improve preoperative risk stratification while still requiring histopathological confirmation in clinically suspicious or discordant cases (9).

The diagnostic value of ultrasound in this study appears to be related to the combined interpretation of grayscale morphology and Doppler vascular distribution rather than reliance on a single sonographic feature. Hypoechoic echogenicity was strongly associated with malignancy, as 30 of 43 hypoechoic lesions were malignant, giving a malignancy proportion of 69.8%, while all non-hypoechoic lesions were benign in this cohort. This is consistent with the established observation that many malignant intratesticular tumors appear hypoechoic because tumor infiltration replaces or distorts normal testicular parenchymal architecture. Ill-defined margins also showed clinically meaningful discriminatory value, with all 30 malignant lesions occurring among the 52 lesions with ill-defined margins, whereas all 23 well-defined lesions were benign. These findings reinforce the importance of careful grayscale assessment because margin irregularity and reduced echogenicity may reflect infiltrative tumor behavior rather than simple mass effect alone (10).

Color Doppler findings provided additional diagnostic information beyond grayscale morphology. Vascularity was present in all 30 malignant lesions and absent in all 22 avascular lesions, indicating that the presence of Doppler flow was a sensitive marker of malignant potential in this cohort. More importantly, vascular pattern showed the strongest discriminatory value: all 30 lesions with central or chaotic vascularity were malignant, whereas all 45 lesions with absent or peripheral vascularity were benign. This pattern is clinically plausible because malignant tumors commonly develop internal vascularity and disorganized neovascular architecture, while benign cystic or inflammatory lesions may show absent flow or predominantly peripheral vascularity. The finding that central or chaotic vascularity showed 100.0% malignancy in this dataset suggests that vascular distribution may be more informative than vascularity alone and should be emphasized in structured ultrasound reporting of testicular lesions (11).

The distribution of histopathological diagnoses in this study also highlights the diverse pathological spectrum encountered in patients with testicular lesions. Benign lesions accounted for 45 of 75 cases, representing 60.0% of the cohort, while malignant lesions accounted for 30 cases, representing 40.0%. Among benign lesions, adenomatoid tumor was the most frequent diagnosis, followed by epididymitis, cyst, and orchitis. Among malignant lesions, non-seminomatous germ cell tumor was the most common histological type, followed by seminoma and lymphoma. This distribution supports the clinical need for accurate preoperative differentiation, because a substantial proportion of clinically or radiologically detected testicular lesions may ultimately be benign. Previous histopathological series have similarly emphasized that non-neoplastic and benign lesions constitute an important proportion of testicular pathology, although the proportion of malignancy may vary according to referral pattern, institutional setting, and inclusion criteria (12).

The diagnostic performance observed in the present study compares favorably with previous regional and international evidence. Earlier studies evaluating color Doppler ultrasound in testicular tumors have reported lower or more variable specificity and diagnostic accuracy, which may reflect differences in lesion spectrum, ultrasound criteria, operator expertise, and whether grayscale findings were interpreted together with Doppler findings. Naz et al. reported sensitivity of 87.0%, specificity of 67.6%, positive predictive value of 71.6%, negative predictive value of 84.7%, and diagnostic accuracy of 77.0% for color Doppler ultrasound in diagnosing testicular tumors, while Fazal et al. reported sensitivity of 88.7%, specificity of 78.1%, positive predictive value of 81.1%, negative predictive value of 86.8%, and accuracy of 83.9% compared with histopathology. The higher accuracy in the present study may be explained by the combined use of grayscale morphology and Doppler vascular pattern, particularly the strong discriminatory effect of central or chaotic vascularity, but this interpretation should be made cautiously because the sample size was modest and the study population consisted of patients selected for histopathological confirmation (13,14).

The clinical probability shift observed in this study further strengthens the practical interpretation of the diagnostic findings. The baseline malignancy prevalence was 40.0%, but a positive combined ultrasound diagnosis increased the post-test probability of malignancy to 93.3%, while a negative ultrasound diagnosis reduced the residual probability of malignancy to 4.4%. This large movement from pre-test to post-test probability indicates that combined ultrasound assessment meaningfully changes clinical risk estimation and may help clinicians identify patients requiring urgent urological evaluation, surgical planning, or histopathological confirmation. At the same time, the presence of 2 false-positive and 2 false-negative cases confirms that ultrasound should not be interpreted as a replacement for histopathology. Instead, ultrasound should be regarded as a high-value first-line triage and characterization tool that improves preoperative decision-making while histopathology remains essential for definitive diagnosis.

Continuous variables, including age, lesion size, and resistive index, did not appear to provide the same discriminatory value as morphology and vascular pattern. The mean lesion size was 27.78 ± 12.38 mm, but lesion size alone did not distinguish benign from malignant pathology in the available analysis. This finding is clinically important because small malignant lesions and larger benign inflammatory or cystic lesions may both occur, making size an unreliable independent criterion for malignancy. Similarly, resistive index did not show clear discriminatory value compared with qualitative vascular pattern. This suggests that the spatial distribution and organization of vascularity may be more clinically meaningful than an isolated Doppler resistance measurement. A multiparametric approach that integrates echogenicity, lesion margin, lesion composition, vascularity, and vascular pattern is therefore more appropriate than reliance on any single ultrasound variable (15).

The findings have important implications for clinical practice in resource-limited settings. Advanced techniques such as contrast-enhanced ultrasound, elastography, and magnetic resonance imaging may provide additional characterization of selected testicular lesions, but these modalities may not be routinely available in many diagnostic centers. Conventional grayscale and color Doppler ultrasound remain more accessible and can provide strong diagnostic information when performed systematically and interpreted using predefined morphological and vascular criteria. In practical terms, lesions that are solid or mixed, hypoechoic, ill-defined, and demonstrate central or chaotic vascularity should be considered high risk and prioritized for specialist urological evaluation and histopathological confirmation. Conversely, lesions with cystic morphology, well-defined margins, absent vascularity, or peripheral vascularity may be more likely to represent benign pathology, although clinical context and follow-up remain important where uncertainty persists (16).

This study has several limitations that should be considered when interpreting the results. First, the sample size was relatively small, with 75 patients and 30 malignant lesions, which may widen uncertainty around diagnostic estimates despite high observed accuracy. Second, the use of non-probability convenience sampling may have introduced selection bias, particularly because only patients with histopathological confirmation were included. This may have increased the proportion of clinically suspicious lesions and may limit generalizability to all patients undergoing routine scrotal ultrasound. Third, the study did not assess interobserver agreement, so the reproducibility of ultrasound interpretation between different operators could not be evaluated. Fourth, although combined grayscale and Doppler features were predefined, operator dependence remains an inherent limitation of ultrasound. Fifth, false-positive and false-negative cases were not separately characterized by histological subtype and imaging pattern, limiting deeper understanding of the causes of diagnostic discordance. Future studies should include larger prospective cohorts, standardized reporting templates, blinded ultrasound interpretation, interobserver reliability assessment, and detailed analysis of discordant cases.

Despite these limitations, the study provides clinically useful evidence that combined grayscale and color Doppler ultrasound has strong diagnostic performance for differentiating benign from malignant testicular lesions when compared with histopathology. The results suggest that vascular pattern, particularly central or chaotic vascularity, may be the most powerful sonographic discriminator of malignancy, while hypoechogenicity and ill-defined margins further strengthen diagnostic suspicion. The high negative predictive value also suggests that ultrasound can be helpful in reducing the probability of malignancy when benign features predominate, although definitive exclusion still depends on clinical judgment and histopathological confirmation when indicated. Overall, these findings support the integration of structured grayscale and Doppler assessment into routine evaluation of testicular lesions and justify further validation in larger, prospective, multicenter studies.

CONCLUSION

Combined grayscale and color Doppler ultrasound demonstrated excellent diagnostic performance in differentiating benign from malignant testicular lesions when compared with histopathological examination as the gold standard. The study found high sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy, with excellent agreement between ultrasound diagnosis and histopathology. Hypoechogenicity, ill-defined margins, presence of vascularity, and especially central or chaotic vascular patterns were strongly associated with malignancy, whereas age, lesion size, and resistive index were not significant discriminators. These findings support the use of combined grayscale and color Doppler ultrasound as an effective first-line imaging modality for preoperative evaluation of testicular lesions, particularly in resource-limited settings, while confirming that histopathology remains essential for definitive diagnosis.

REFERENCES

1. Boschian R, Smarrazzo L, Guido A, Cevenini G, Rossi M. Focal testicular lesions: multiparametric US features and association with histopathology. J Urol. 2019;201.

2. Isidori AM, Pozza C, De Matteis A, Mainini G, Montanari M, Ferrari ME, et al. Differential diagnosis of nonpalpable testicular lesions: qualitative and quantitative contrast-enhanced ultrasound of benign and malignant testicular tumors. Radiology. 2014;273(2):606-18.

3. Auer T, Solà EJV, Auer-Flöckl AM, Habermann CR, Grosse U, Naguib NN, et al. Value of multiparametric ultrasound in the assessment of intratesticular lesions. Radiology. 2017;285(2):640-9.

4. Mannaerts CK, Cagiannos I, Zappavigna C. Contrast-enhanced ultrasound. In: Rastinehad AR, editor. Interventional Urology. Cham: Springer International Publishing; 2021. p. 161-77.

5. Elzinga-Tinke JE, Dohle GR, Looijenga LHJ. Etiology and early pathogenesis of malignant testicular germ cell tumors: towards possibilities for preinvasive diagnosis. Asian J Androl. 2015;17(3):381-93.

6. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. BMJ. 2015;351.

7. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.

8. Dogra VS, Gottarelli G, Rubens DJ, Liao L. Sonography of the scrotum. Radiology. 2003;227(1):18-36.

9. Mirochnik B, Bhatt S, Dogra VS. Ultrasound evaluation of scrotal pathology. Radiol Clin North Am. 2012;50(2):317-32.

10. Belfield J, Findlay-Line C. Testicular germ cell tumours: the role of conventional ultrasound. Cancers (Basel). 2022;14(16):3882.

11. Pozza C, Deininger S, Kranz J, Mucelli RP, Pozza F, Thouvenin Y, et al. Multiparametric ultrasound for diagnosing testicular lesions: everything you need to know in daily clinical practice. Cancers (Basel). 2023;15(22):5332.

12. Baidya R, Sigdel B, Baidya N. Histopathological pattern of testicular lesion. J Pathol Nepal. 2017;7(1):1087-90.

13. Naz N, Khan NA, Hotchandani K. Diagnostic accuracy of color Doppler ultrasonography to diagnose testicular tumors. J Fatima Jinnah Med Univ. 2018;12(2):74-7.

14. Fazal K, Khan I, Khan S, Bilal M, Amin M. Color Doppler ultrasound for diagnosis of testicular carcinoma: a comparison with gold standard histopathology. Ann Med Surg (Lond). 2022;84:104938.

15. Reginelli A, Gassino D, Urraro F, Maione L, Belfiore G, Grassi R, et al. Does multiparametric US improve diagnostic accuracy in the characterization of small testicular masses? Gland Surg. 2019;8(Suppl 3).

16. Almassry HN, Elsaid HH, Alhawary TB, Assem MA. The validity of grayscale and color Doppler ultrasound in assessment of scrotal swellings: a retrospective study in a large case series. Acta Radiol. 2021;62(2):266-75.

17. Ali TZ, Parwani AV. Benign and malignant neoplasms of the testis and paratesticular tissue. Surg Pathol Clin. 2009;2(1):61-159.

18. Rizvi SAA, Hussain M, Manzoor K, Hasan A, Shah S, Ali G. Role of color Doppler ultrasonography in evaluation of scrotal swellings: pattern of disease in 120 patients with review of literature. Urol J. 2011;8(1):60-5.

19. Lewicki A, Advani Z, Banz VM, Castelain M, Casula V, Côté GA, et al. Incidental findings and how to manage them: testis—a WFUMB position paper. Ultrasound Med Biol. 2021;47(10):2787-802.

20. Rocher L, Muller J, Jouve JL, Perol M, Garcia M, Delsaux C, et al. Incidentally detected non-palpable testicular tumours in adults at scrotal ultrasound: impact of radiological findings on management. Radiologic review and recommendations of the ESUR scrotal imaging subcommittee. Eur Radiol. 2016;26(7):2268-78.

21. Butruille C, Czernin M, Bouchot A, Tostain G. Management of testicular lesions in a population of infertile patients. Prog Urol. 2012;22(1):45-52.

22. Bahrami A, Ro JY, Ayala AG. An overview of testicular germ cell tumors. Arch Pathol Lab Med. 2007;131(8):1267-80.

23. Mittal PK, Abdalla AS, Chatterjee A, Baumgarten DA, Harri PA, Patel J, et al. Spectrum of extratesticular and testicular pathologic conditions at scrotal MR imaging. Radiographics. 2018;38(3):806-30.

24. Patrikidou A, Ruf C, Kremastinos D, Eckardt K, Weissbach L, Sedlmayer F, et al. European Association of Urology guidelines on testicular cancer: 2023 update. Eur Urol. 2023;84(3):289-301.

25. Dieckmann KP, Skakkebaek NE. Carcinoma in situ of the testis: review of biological and clinical features. Int J Cancer. 1999;83(6):815-22.

26. Xue N, Zhang S, Wang G. The value of contrast-enhanced ultrasonography in the diagnosis of primary testicular non-neoplastic and neoplastic lesions in adults. BMC Urol. 2022;22(1):210.

27. Huang DY, Yarmohammadi H, Alsabti H, Schlossberg S, Hripcsak G, Dogra VS, et al. Multiparametric ultrasound for focal testicular pathology: a ten-year retrospective review. Cancers (Basel). 2024;16(13):2309.

28. Naeem MA, Khan M, Bilal M, Amin M, Shafiq K. Diagnostic accuracy of color Doppler and grey scale ultrasonography in the diagnosis of painful scrotal conditions. Ophthalmol Update. 2021;19(3):192-6.

29. Pinto J, Patil P, Paixão D, Sharma R, Batra R, Sidhu PS. Contrast-enhanced ultrasound in testicular focal lesions: a systematic review. Eur Radiol. 2021;31(11):8991-9002.