Comparison of Conventional Ultrasound, Doppler, Elastography and Contrast Enhanced Ultrasonography Parameters with Histopathology Findings in the Differential Diagnosis of Benign and Malignant Thyroid Nodules

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Abstract

Background: Though neck ultrasound is the first line of choice for the screening of thyroid nodules, very few studies have compared the diagnostic performances of both conventional and advanced ultrasound parameters. In this study, we aim to compare various conventional and advanced ultrasound imaging parameters and confirm it with histopathological findings to differentiate between benign and malignant thyroid nodules.

Methods: One hundred and thirty nine patients with 173 thyroid nodules underwent conventional ultrasonography (cUSG) which included gray-scale parameters, colour Doppler (CD) and power Doppler (PD) followed by elastography and contrast enhanced ultrasonography (CEUSG). Post-USG imaging all patients underwent fine needle aspiration cytology followed by surgery if indicated and histopathological results were obtained. Stata 11.2 statistical software was used for the statistical analysis.

Results: Of 173 nodules, 65 were benign and 108 were malignant. cUSG had a sensitivity, specificity, PPV, NPV and accuracy of 94.4%, 90.4%, 94.4%, 90.4% and 91.9%, respectively with AUC:0.97. On ROC analysis, the cut-off value for differentiating malignant from benign thyroid nodules on Ueno elasticity scoring was >3; AUC: 0.86 versus >2.2 using elasticity ratio method, AUC: 0.90. CEUSG and elastography had sensitivity, specificity, PPV, NPV and accuracy of 93.8%, 95.3%, 97.2%, 89.8% and 94.2%, respectively with AUC:0.98. On combining and ranking, both conventional and advanced cUSG parameters, the significant indicators for malignancy were heterogeneous contrast enhancement, followed by Type-IV/V PD flow patterns, absence of ring enhancement and elasticity ratio >2.2 patterns with the largest AUC:0.904.

Conclusions: Conjoint analysis of specific features of thyroid nodules on cUSG, elastography and CEUSG will enhance the diagnostic value in the screening of thyroid nodules.

Keywords: Ultrasound; Fine needle aspiration cytology; Histology; Thyroid nodules

Introduction

Thyroid nodules may present as various types such as nodular hyperplasia, adenomas, autoimmune disease and carcinoma. Only 7% of these nodules are malignant of which 5% are present in females and 1% in males [1,2]. The incidence of thyroid cancer has increased from 4.9 per 100000 to 14.3 per 100000 in 2009 [3]. Conventional ultrasonography (cUSG) is generally considered as the choice of investigation of thyroid nodules. ATA guidelines strongly recommend thyroid USG to be performed for screening in all patients with known or suspicious thyroid nodules. CUSG provides information regarding the size, echogenicity pattern, nodule component, blood flow pattern and suspicious cervical lymphadenopathy if present [4]. The pattern of nodule helps in the decision making of fine needle aspiration cytology (FNAC). FNAC is considered the gold standard cost-effective and most accurate method in evaluating thyroid nodules. However, there is a higher likelihood of false negative cases due to non-diagnostic cytology and cystic component [4]. Hence, USG guided FNAC is favoured over blinded FNAC [5,6]. However, due to the low sensitivity and specificity of cUSG, newer techniques such as elastography [7-9] and contrast enhanced ultrasonography (CEUSG) [10] have been developed to improve the accuracy of ultrasound in differentiating benign from malignant nodules. Real-time tissues elastography techniques have recently undergone extensive research and have gained importance in differentiating benign from malignant thyroid nodules. The principle of elastography is based on tissue elasticity. Tissue compression causes deformation and displacement of the tissue. Lesser displacement and lesser strain are observed in harder tissues compared to softer tissues. Qualitative elastography is based on an elastogram displayed using a polychromatic scale ranging from red to blue depending on the strain of the tissue and is scored between 1 to 5 [11]. However, the scoring is largely influenced by intra and inter-observer variability. To compensate for such variabilities, quantitative elastographic tools such as elasticity ratio (ER) [12] and strain index (SI) [13] have been developed and have proved beneficial to measure the elasticity in tissue. CEUSG is a

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new emerging technique which facilitates the study of microvascular perfusion of the nodules and overcomes the limitations of imaging [10]. As there are limited studies reported in literature which compare the diagnostic performances of both conventional and advanced ultrasound parameters, we aimed to compare various cUSG, elastography and CEUSG features in differentiating benign and malignant nodules.

Material and Methods

Place of study
The present study is a prospective analysis, involving patients who presented with thyroid nodules and were evaluated from 2014 to 2016 at our hospital. The study was approved by the institute ethics committee and informed consent was obtained from all the patients.

Patient recruitment
All India Institute of Medical Sciences is a tertiary centre delivering health care to the whole of northern India covering half-a-billion people with a high volume of surgical referrals. Majority of the patients referred were previously explored in other hospitals with strong suspicion of thyroid malignancy to rule in or rule out disease.

Patient inclusion and exclusion criteria
Patients having single or multiple thyroid nodules on examination or imaging irrespective of biochemical status were selected. Patients were referred from surgical departments or centres with previously performed Bethesda I or Bethesda III FNAC’s which needed repeat procedures. Patients with high suspicion for malignancy on imaging and clinical features were also included. Only patients who gave informed consent were only included in the study. Patients with a previous history of thyroid surgery, hypersensitivity to sulphur hexafluoride and coronary artery disease were excluded from the study.

One hundred forty four consecutive patients were recruited for the study. Two patients with sulphur hexafluoride hypersensitivity and three patients with prior cytology report were excluded. Finally, a total of 173 thyroid nodules in 139 patients were included in this prospective study. The patients with follicular neoplasm underwent hemithyroidectomy. The patients with proven papillary carcinoma thyroid underwent near total or total thyroidectomy (NTT) along with lymph node dissection if positive for lymph node on imaging or clinical features. Patients with prior cytology report were excluded. Finally, a total of 173 thyroid nodules in 139 patients were included in this prospective study. The patients with follicular neoplasm underwent hemithyroidectomy. The patients with proven papillary carcinoma thyroid underwent near total or total thyroidectomy (NTT) along with lymph node dissection if positive for lymph node on imaging or clinical features. Patients suspicious for malignancy were subjected to either NTT or lobectomy depending on the size of the nodule.

Equipment
Conventional B-mode, colour Doppler and power Doppler, elastography and CEUSG examination in all patients was performed on a prototype LOGIQ E9 US system using a multi linear probe with frequency range of 6-15 MHz (GE Healthcare, Chalfont St. Giles, UK). All patient scans were performed and interpreted by a single ultrasonologist in order to minimize inter-observer variability. The ultrasonologist performing and interpreting the scans was blinded to the results of FNAC findings and other anatomical imaging reports if performed before USG.

Conventional ultrasonography
Conventional USG include gray-scale USG, colour Doppler, power Doppler was performed in all thyroid nodules by maintaining maximum image quality. The thyroid nodules were evaluated and classified as benign or malignant on various morphological aspects of the nodule as shown in Table 1.

Colour Doppler ultrasound
The colour Doppler imaging was performed to evaluate the type of vascularity of the thyroid nodule for predicting malignancy. The type of flow pattern was classified accordingly; Type-I-absence of blood flow, Type-II-marked perinodular with or without slight intranodular blood flow, Type-III-marked intranodular with or without slight perinodular blood flow.

Power Doppler ultrasound
In comparison with colour Doppler, power Doppler is more sensitive in the detection of low volume flow and is not dependent on the direction of the flow. Therefore, power Doppler was performed in all patients and blood flow patterns were classified accordingly. Type-I-absence of blood flow, Type-II-exclusively perinodular blood flow, Type-III-perinodular blood flow ≥ central blood flow, Type-IV-marked central blood flow with less peripheral blood flow and Type-V-exclusively marked intranodular blood flow. The blood flow patterns as defined by Channas et al. were adopted in the current study [14].

Elastography
Real-time elastography was performed using a 6-15ML MHz high frequency multi linear probe by applying repeated period quasistatic compression and decompression technique in vertical direction. The dynamic data was stored in frames and used for analysis using the q-analysis software. The elastographic image was overlaid on the B-mode USG image with a colour scale ranging from blue which codes organs with maximum strain to red for organs with no strain. The colour code of the nodule was matched and classified according to the Ueno & Ito elasticity score [11]. According to their scoring, score 1 indicated elasticity in the entire nodule (strain in the entire lesion), score 2-elasticity in large part of the nodule (strain in large area of the lesion), score 3- elasticity in only peripheral part of the nodule (strain in the periphery of the lesion), score 4- no elasticity in the entire nodule (no strain in the entire lesion) and score 5-no elasticity in the entire nodule and posterior shadowing (no strain in the entire lesion and the surrounding area). Quantitative parameters like elasticity index (EI) and elasticity ratio (ER) were also analysed. Elasticity index is defined as the relative hardness of tissues ranging from 0.0 to 6.0 which is obtained by drawing a ROI in the saved dynamic images. A higher value indicates higher stiffness. Real time elastographic images were stored as dynamic images in frames in 0.1-s interval timings. From the motion images, elasticity index was calculated as a mean value from all the dynamic frames. Within an elastographic image of thyroid a mean elasticity index EI was defined by drawing a circular ROI of 8 mm on normal thyroid tissue. EI values were considered as the reference standard. Elasticity ratio was calculated as the elasticity index of the inclusion (abnormal tissue) divided by the EI of reference (EI) (E2/E1) (12).

Contrast enhanced ultrasonography imaging technique and analysis
All the CEUSGs were performed by a single operator and was blinded to the diagnosis. Initially, conventional ultrasonography was done to localize the thyroid nodule in transverse section. The localization was done is such a way that both abnormal thyroid tissue and normal parenchyma were visualised along with the carotid artery and jugular vein. The mechanical index was kept <0.13 to prevent microbubble destruction. The sulfur hexafluoride microbubble contrast medium, Sonovue (Bracco, Milan, Italy) was used. The contrast agent was diluted with 5 ml of 0.9% w/v sodium chloride solution followed by vigorous
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shaking which resulted in a milky white solution due to the production of microbubbles of sulphur hexafluoride. The mean diameter of the microbubbles was 2.5 µm. The patients were manually injected with 2.5 ml Sonovue contrast intravenously at a rate of 1ml/sec, followed by 10 ml of normal saline through the cubital vein. In the meantime, the contrast image acquisition was started using timer and all the dynamic raw data were stored in cine loop up to 2 minutes. The position of the transducer was kept immobilised during the acquisition in order to minimise motion artefacts.

Qualitatively, the thyroid nodules were evaluated using various criteria. Criteria 1: relative arrival time of contrast with respect to normal parenchyma; earlier or same time and delayed time. Criteria 2: distribution of contrast enhancement of the lesion with respect to normal parenchyma; homogenous enhancement, heterogeneous enhancement, ring enhancement and no enhancement. Criteria 3: on the basis of type of enhancement of contrast agent with respect to normal parenchyma; isoechoic, hypoechoic, heteroechoic and anechoic enhancement.

The offline quantitative analysis was performed using software “Time intensity curve analysis” (TIC) where two regions of interest were drawn manually on the CEUSG image and enabled in all frames of the acquisition loop. First, ROI was drawn in the tumour. Second ROI was drawn in the adjacent normal parenchyma at the same depth with tumour followed by which a time intensity curve was derived by the

<table>
<thead>
<tr>
<th>Variables</th>
<th>Benign Nodules n=65</th>
<th>Malignant nodules N=108</th>
<th>p-value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
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<tr>
<td>Calcifications</td>
<td>Microcalcification</td>
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<td>Component</td>
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<td>52.3%</td>
<td>71%</td>
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<td>Echogenicity</td>
<td>Isoechoic/hyperchoic</td>
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<td>Halo Complete</td>
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<td>78.9%</td>
<td>84.4%</td>
<td>38.5%</td>
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<td>Margins Regular</td>
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<td>70.3%</td>
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<td>Colour Doppler Type -I/III</td>
<td>50</td>
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<td>76.9%</td>
<td>83.8%</td>
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<td>78.2%</td>
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<td>Type -III</td>
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<td>Power Doppler Type-II/III</td>
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<td>93.4%</td>
<td>71.9%</td>
<td>85.3%</td>
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<td>Type -IV/IV</td>
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<td>85</td>
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<td>Ring enhancement Present</td>
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<td>Absent</td>
<td>19</td>
<td>103</td>
<td>&lt;0.0001</td>
<td>95.3%</td>
<td>70.7%</td>
<td>84.4%</td>
<td>90.2%</td>
<td>86.1%</td>
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<td>Arrival of contrast Early/same time</td>
<td>58</td>
<td>27</td>
<td>&lt;0.0001</td>
<td>75%</td>
<td>89.2%</td>
<td>92%</td>
<td>68.2%</td>
<td>81.3%</td>
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<td>Delayed</td>
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<td>81</td>
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<tr>
<td>Type of contrast enhancement Iso/hyper/no enhancement</td>
<td>63</td>
<td>19</td>
<td>&lt;0.0001</td>
<td>82.4%</td>
<td>96.9%</td>
<td>97.8%</td>
<td>76.8%</td>
<td>89.3%</td>
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<td>Hypo</td>
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<tr>
<td>Distribution of contrast enhancement Homogenous</td>
<td>60</td>
<td>8</td>
<td>&lt;0.0001</td>
<td>92.5%</td>
<td>93.2%</td>
<td>95.2%</td>
<td>88.2%</td>
<td>92.4%</td>
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<tr>
<td>Heterogeneous</td>
<td>5</td>
<td>100</td>
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<tr>
<td>UENO Elasticity score Score 1/2/3</td>
<td>48</td>
<td>8</td>
<td>&lt;0.0001</td>
<td>92.5%</td>
<td>73.8%</td>
<td>85.4%</td>
<td>85.7%</td>
<td>85.5%</td>
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<tr>
<td>Score 4/5</td>
<td>17</td>
<td>100</td>
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<tr>
<td>Elasticity Index (mean ± SD)</td>
<td>1.96 ± 0.98</td>
<td>6.52 ± 1.32</td>
<td>&lt;0.0001</td>
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<td>Elasticity ratio(mean ± SD)</td>
<td>1.388 ± 1.3</td>
<td>5.19 ± 2.6</td>
<td>&lt;0.0001</td>
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<tr>
<td>Time to peak (mean ± SD)</td>
<td>17.77 ± 6.6</td>
<td>19.21 ± 1.3</td>
<td>0.323</td>
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<td></td>
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<tr>
<td>Maximum intensity (Imax) (mean ± SD)</td>
<td>54 ± 5.4</td>
<td>42 ± 4.8</td>
<td>&lt;0.0001</td>
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</table>

PPV: Positive Predictive Value; NPV: Negative Predictive Value; SD: Standard Deviation.

Table 1: Comparison of various conventional and advanced ultrasonography features between benign and malignant thyroid nodules.
software and 2 perfusion parameters were extracted, including time-to-peak (TTP, time of beginning of arterial enhancement until the peak enhancement of contrast) and maximum intensity of peak in decibels (I_{max}).

**Fine needle aspiration cytology**

USG guided FNAC was done in all the thyroid nodules. The FNAC results were categorized according to the Bethesda system for reporting thyroid cytopathology [15].

**Histological analysis**

Nodules with suspicious or proven carcinoma on FNAC underwent surgery. Certain benign nodules with large size causing breathlessness were also operated. Histological analysis was considered the gold standard for differentiating benign and malignant thyroid nodules. The histopathological data were evaluated and categorized according to the World Health Organization histological classification of tumors [16].

**Statistical analysis**

Univariate analysis was used to compare the various ultrasound features between benign and malignant thyroid nodules. Continuous variables were calculated as mean, range and standard deviation (SD) and compared using Student's t-test. Categorical variables were compared by chi-square test or the Fisher's exact test on the basis of expected cell frequencies. Multivariate logistic regression analysis was applied to derive valuable predictive factors according to the odds ratio (OR). The cut-off for elastography techniques were derived by receiver operating curve analysis (ROC) analysis. p-values ≤ 0.05 were considered as significant. Stata 11.2 (StataCorp, College Station, TX, USA) was used for the analysis.

**Results**

One-hundred and thirty nine patients with 173 thyroid nodules (mean age ± SD: 39.3 ± 17.47 years; range: 6-75 years), were enrolled in the study. The female to male ratio was 3.8 in our study.

**Comparison of FNAC with histopathology results**

Solitary thyroid nodules were present in 103 patients and multinodular goitre in 36 patients summing up to 173 thyroid nodules. USG guided FNAC was performed for all 173 thyroid nodules. Based on FNAC results, 11 nodules comprised of Bethesda category I [BC-I], 19 adenomatous goitres (BC-II), 4 lymphoctic thyroiditis (BC-III), 35 follicular neoplasms (BC-IV), 18 suspicious nodules for malignancy (BC-V), and 86 malignant nodules (BC-VI). Twenty two of 34 nodules with benign cytology, all 53 suspicious nodules and 86 nodules with malignant cytology were operated. The malignant and benign cytology results were compared with the post-operative histopathological findings. 100% concordance was observed between FNAC and histopathology results in both benign and malignant nodules. Regarding, BC: IV and V nodules, postoperative malignancy FNAC and histopathology results in both benign and malignant histopathological findings. 100% concordance was observed between conventional ultrasound parameters, Type-I/IV-V vascularisation pattern on power Doppler had the highest specificity with OR 130.34; 95% CI: 20.231 – 839.76 (sensitivity: 78.7; specificity: 90.2%; PPV: 93.4%; NPV: 71.9%; accuracy: 85.3%), followed by hypoechogenity (OR: 46.9; 95% CI: 4.472 – 492.30) and microcalcifications (OR:26.43; 95% CI: 5.321-131.29). cUSG had a sensitivity, specificity, PPV, NPV and accuracy of 94.4%, 90.4%, 94.4%, 90.48%, and 91.9%, respectively with AUC:0.97 (95% CI: 0.942-0.994).

**Elastography**

Of the 65 benign nodules, 48 nodules had a score of 1 or 2, 11 had a score of 3 and 6 patients had a score of 4 or 5 (3 nodules had a score of 4 and 3 had a score of 5). Of 108 malignant nodules, 100 nodules had a score of 4 or 5 (81 nodules: score 4 and remaining 19 nodules had a score of 5). 8 malignant nodules had a score of 2. On further analysis of follicular neoplasms, 25 of 35 nodules had a score of 2, 6 had a score of 3 and the remaining 4 had a score of 4. The sensitivity, specificity PPV NPV and accuracy of elasticity score in the differential diagnosis of benign and malignant nodules are 92.5%, 73.5%, 85.4%, 85.75% and 85.5%, respectively with AUC: 0.86. Quantitative parameters such as elasticity index (EI) and elasticity ratios (ER) were also assessed in all the nodules. There was a significant difference in the mean elasticity index and elasticity ratio between benign and malignant nodules (Table 1). On ROC analysis the AUC for differentiating malignant from benign thyroid nodules on Ueno elastography scoring was 0.86 (sensitivity=92.6%, specificity=82.4%) with a cut-off of >3. As per the literature a known cut-off of >2.3 from literature was applied for elasticity ratio evaluation [17]. Using the ROC analysis, the best cut-off value obtained using elasticity ratio method was >2.2. The area under the ROC curve for elasticity ratio was 0.90 (95% CI: 0.85-0.94, sensitivity: 85.6%, specificity: 92.7%) (Figure 2).

**Contrast enhanced ultrasonography**

Various qualitative and quantitative CEUSG parameters for the differential diagnosis of benign and malignant nodules are shown in Table 1. Among the qualitative parameters heterogeneous, hypoechogenic enhancement and delayed arrival of contrast with respect to normal parenchyma were features favouring malignant thyroid nodules and were significantly different between the benign and malignant thyroid nodules (Table 1, Figures 3 and 6). The hypoechogenic contrast enhancement had the highest specificity of 96.9% followed by heterogeneous enhancement of contrast 93.2% for differentiating malignant from benign thyroid nodules (Table 1). On logistic regression analysis, among CEUSG and elastography parameters, hypoechogenic contrast enhancement pattern in malignant nodules had the highest OR of 108.6 (95% CI: 10.64-1109) followed by heterogeneous contrast enhancement with OR 21.33 (95% CI: 4.38-100.89). Ring enhancement in benign nodules, OR: 58.55 (95% CI: 5.906-580.4) was the strongest predictor for benign nodules (Table 2, Figures 4 and 6). CEUSG and elastography had a sensitivity, specificity, PPV, NPV and accuracy of 94.4%, 90.4%, 94.4%, 90.48%, and 91.9%, respectively with AUC:0.97 (95% CI: 0.942-0.994).
For follicular neoplasms (Figure 5, Figure 6B and 6D) and nodular goitres (Figure 6E and 6F) there was no difference in the peak intensity value with respect to surrounding normal thyroid tissue. Table 1 shows the comparison quantitative parameters of the TIC between benign and malignant nodules. There was no significant difference in time to peak between benign and malignant nodules. However, a significant difference in the Max intensity was observed (p<0.0001).

cUSG, elastography and CEUSG features were combined and ranking of valuable indicators were done using logistic regression analysis. The significant indicators for malignancy were heterogeneous.
Discussion

The recent ATA guidelines recommend USG as the first line imaging choice for the screening of thyroid nodules [4]. Many technical advances have developed in the field of USG to accurately diagnose and differentiate the benign and malignant thyroid nodules. In this study, we aim to discuss the comparison of various conventional, elastographic and contrast enhancement features in differentiating benign and malignant thyroid nodules. In the current study, all the patients underwent USG guided FNAC to avoid false negative cases [17]. The malignancy rate in our study was strikingly high, 49.7% cytologically [17]. The area under the curve for diagnosing malignant nodule by Ueno elasticity scoring system was 0.86. The results agree with Xing [29] and elevated sensitivity and specificity of 92.3% and 88% respectively [14]. Unfortunately, there are limited numbers of studies evaluating the significance of power Doppler in the assessment of thyroid nodules. We observed Type-IV power Doppler based blood flow pattern and microcalcifications as the most specific combination in predicting malignancy. Consistent with our results, Rago et al. found microcalcifications with marked intranodular blood flow as the most specific combination in predicting malignancy [26]. Similarly, Gul et al. (sensitivity 65.2%, specificity 98.7%, PPV 71.6%) and Asteria et al. (sensitivity 41%, specificity 99%) reported microcalcifications and hypoechoogenicity as the most predictive combination in detecting malignancy [8,21]. The sensitivities and specificities vary widely among different studies (Table 4) and no single conventional ultrasonography parameter is 100% specific for malignancy. When various conventional USG parameters were combined and ranked with advanced ultrasonography parameters, only Type-IV/V power Doppler vascularity pattern remained as second strongest indicator in predicting malignancy (OR: 283.17 95% CI:15.10 to 5380) (Table 3). In this study cUSG had a sensitivity of 94.4%, specificity 90.4% and accuracy of 91.9%. However, compared with our results, Shuizen et al. reported a higher sensitivity, lower specificity and lower accuracy of 97%, 6.9% and 35.1%, respectively [27].

Elastography measures the tissue elasticity by distortion of the tissue structures using external pressure. The degree of distortion reflects the hardness between different tissues, malignant and benign lesions. The differences are displayed as a colour scale; soft tissue is shown as blue-green and hard tissue as red. Since most of the malignant tumours are hard with decreased elasticity, they usually represent red colour on the colour scale. Ueno et al. [11] have scaled from 1-5 and Rubaltelli et al. have scaled between 0-4 [28]. We chose the 5 colour scale pattern as proposed by Ueno et al. In our study 100/108 (92.5%) malignant nodules had a score of 4 or 5; 48/65 (73.8%) benign nodules had a score of 1 to 2.

Table 2

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>SE</th>
<th>p-value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterogeneous Contrast enhancement</td>
<td>1.330</td>
<td>0.0014</td>
<td>70.96</td>
<td>5.230-962.38</td>
</tr>
<tr>
<td>Hypoechoic contrast enhancement pattern</td>
<td>4.407</td>
<td>0.0007</td>
<td>82.08</td>
<td>6.474-1040</td>
</tr>
<tr>
<td>Ring enhancement of contract</td>
<td>1.591</td>
<td>0.0154</td>
<td>47.30</td>
<td>2.090-1070</td>
</tr>
<tr>
<td>Elasticity ratio</td>
<td>1.386</td>
<td>0.0082</td>
<td>39.07</td>
<td>2.582-591.30</td>
</tr>
</tbody>
</table>

SE: Standard Error; OR: Odds Ratio; 95% CI: 95% Confidence Interval.

Figure 6: Comparison of contrast enhanced TIC curves in different thyroid nodules. (A,B) contrast enhanced image and TIC of papillary thyroid carcinoma nodule: the green curve represents the lesion and the yellow curve represents the normal thyroid tissue. (C,D) depict contrast enhanced image and TIC of follicular adenoma; the yellow curve represents the lesion and red represents the normal thyroid tissue. (E,F) depict contrast enhanced image and TIC of nodular goitre; the green and aqua green curves represent signify the nodule and the orange curve represents normal tissue. Papillary thyroid carcinoma is characterised by low peak intensity value than the surrounding normal tissue (B), whereas follicular adenomas (D) and nodular goitres (F) have same/higher peak intensity value compared to surrounding normal thyroid tissue.
Rubaltelli et al. [28]. We also found that the elasticity ratios were different between benign and malignant nodule. On evaluating this method by ROC analysis we observed a best cut-off of >2.2 for differentiating malignant lesions (sensitivity: 85.2%, specificity: 97.1%, AUC:0.90). The elasticity ratio method had a better diagnostic performance than the Ueno elasticity scoring system (Figure 2). Consistent with our results, Abdelrahaman and his group reported a similar cut-off of >2.3 strain ratio predictive for malignancy [17].

In this study we found an overlap of elasticity between benign and malignant thyroid nodules. The Ueno elasticity scoring of 6 post-operative benign lesions were found to be 4/5 and their elasticity ratios were greater than 2.2. Four among them were completely cystic nodules with haemorrhage and the remaining 2 nodules were lymphocytic thyroiditis which might have altered the stiffness due to disappearance of normal follicular epithelium and replacement with lymphocytes and plasma cell infiltration and fibrosis. Consistently, Xing et al. also observed a similar elasticity between benign and malignant nodules. He observed clustered microcalcifications and lymphocytic thyroiditis in such nodules [29].

Strikingly, we also observed 8 of 108 malignant nodules score 2 on Ueno elasticity scoring, but on the contrary had a mean elasticity score of 3.3. FNAC findings revealed follicular neoplasms in all 8 nodules. The higher elasticity ratio among these nodules was probably due to the stage of formation/development of the neoplasm. Quantitative elasticity score method may probably be a better choice for evaluating follicular neoplasms.

Extensive research has been conducted on various gray scale ultrasound and elastography parameters in differentiating thyroid nodules. However, very few groups have discussed the role of CEUSG in differentiating malignant from benign thyroid nodules; still have failed to compare both conventional and other advanced ultrasound parameters with CEUSG [31-34]. The perfusion of Sonovue contrast agent in an organ depends on the microvascularization. Normal thyroid gland has a uniform enhancement after intravenous injection of Sonovue contrast agent whereas, thyroid nodules have different parameters with CEUSG [31-34]. The perfusion of Sonovue contrast agent in an organ depends on the microvascularization. Normal thyroid gland has a uniform enhancement after intravenous injection of Sonovue contrast agent whereas, thyroid nodules have different parameters with CEUSG [31-34]. The perfusion of Sonovue contrast agent in an organ depends on the microvascularization. Normal thyroid gland has a uniform enhancement after intravenous injection of Sonovue contrast agent whereas, thyroid nodules have different parameters with CEUSG [31-34]. The perfusion of Sonovue contrast agent in an organ depends on the microvascularization. Normal thyroid gland has a uniform enhancement after intravenous injection of Sonovue contrast agent whereas, thyroid nodules have different parameters with CEUSG [31-34]. The perfusion of Sonovue contrast agent in an organ depends on the microvascularization. Normal thyroid gland has a uniform enhancement after intravenous injection of Sonovue contrast agent whereas, thyroid nodules have different parameters with CEUSG [31-34]. The perfusion of Sonovue contrast agent in an organ depends on the microvascularization. Normal thyroid gland has a uniform enhancement after intravenous injection of Sonovue contrast agent whereas, thyroid nodules have different parameters with CEUSG [31-34]. The perfusion of Sonovue contrast agent in an organ depends on the microvascularization. Normal thyroid gland has a uniform enhancement after intravenous injection of Sonovue contrast agent whereas, thyroid nodules have different parameters with CEUSG [31-34]. The perfusion of Sonovue contrast agent in an organ depends on the microvascularization. Normal thyroid gland has a uniform enhancement after intravenous injection of Sonovue contrast agent whereas, thyroid nodules have different parameters with CEUSG [31-34]. The perfusion of Sonovue contrast agent in an organ depends on the microvascularization. Normal thyroid gland has a uniform enhancement after intravenous injection of Sonovue contrast agent whereas, thyroid nodules have different parameters with CEUSG [31-34]. The perfusion of Sonovue contrast agent in an organ depends on the microvascularization. Normal thyroid gland has a uniform enhancement after intravenous injection of Sonovue contrast agent whereas, thyroid nodules have different parameters with CEUSG [31-34].

The actual task lies in the evaluation of follicular neoplasms. Our study revealed majority of follicular neoplasms to have a Ueno elasticity score of 2 with few of them scoring 4. The large variation may be due to the stage of formation/development of the neoplasm. Quantitative elasticity score method may probably be a better choice for evaluating follicular neoplasms.

Table 4: Comparison of various conventional ultrasound variables in predicting malignancy according to previous studies and present study.

<table>
<thead>
<tr>
<th>Author</th>
<th>N</th>
<th>Microcalcifications</th>
<th>Halo</th>
<th>Echogenicity</th>
<th>Margins</th>
<th>Blood flow patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rago et al. (7)</td>
<td>92</td>
<td>SE=64% SP=72%</td>
<td>SE=61% SP=82%</td>
<td>SE=81% SP=62%</td>
<td>SE&gt;= SP=</td>
<td>SE=6.4% SP=97%</td>
</tr>
<tr>
<td>Gul et al. (8)</td>
<td>3404</td>
<td>SE=85.7% SP=98.2%</td>
<td>SE=98% SP=30.6%</td>
<td>SE=86% SP=67%</td>
<td>SE=90.2% SP=87.3%</td>
<td>SE=-- SP=</td>
</tr>
<tr>
<td>Sipo et al. (9)</td>
<td>-</td>
<td>SE=42.3% SP=91.2%</td>
<td>SE=83.6% SP=61.2%</td>
<td>SE=53% SP=73.2%</td>
<td>SE=57.7% SP=85.1%</td>
<td>SE=77.2% SP=79.3%</td>
</tr>
<tr>
<td>Ma et al. (20)</td>
<td>172</td>
<td>SE=66% SP=94.9%</td>
<td>SE=95.7% SP=51.3%</td>
<td>SE=77.7% SP=79.5%</td>
<td>SE=-- SP=</td>
<td>SE=-- SP=</td>
</tr>
<tr>
<td>Asterolia et al. (21)</td>
<td>87</td>
<td>SE=59% SP=84%</td>
<td>SE=100% SP=14%</td>
<td>SE=65% SP=81%</td>
<td>SE=76% SP=78%</td>
<td>SE=-- SP=</td>
</tr>
<tr>
<td>Wang et al. (22)</td>
<td>51</td>
<td>SE=66% SP=75%</td>
<td>SE=-- SP=</td>
<td>SE=27-87% SP=74-94%</td>
<td>SE=17-78% SP=39-85%</td>
<td>SE=54-74% SP=79-81%</td>
</tr>
<tr>
<td>Rago et al. (23)</td>
<td>-</td>
<td>SE=59% SP=86 – 90%</td>
<td>SE= SP=</td>
<td>SE=86% SP=71%</td>
<td>SE=92% SP=84%</td>
<td>SE=35% SP=58%</td>
</tr>
<tr>
<td>Hong et al. (24)</td>
<td>145</td>
<td>SE=55% SP=94%</td>
<td>SE= SP=</td>
<td>SE=57% SP=72%</td>
<td>SE=62% SP=93%</td>
<td>SE=54% SP=28%</td>
</tr>
<tr>
<td>Vito et al. (25)</td>
<td>97</td>
<td>SE=27% SP=78%</td>
<td>SE= SP=</td>
<td>SE=66% SP=83%</td>
<td>SE=64.8% SP=76.9%</td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>173</td>
<td>SE=64.8% SP=86.3%</td>
<td>SE=93.5% SP=58.5%</td>
<td>SE=98.1% SP=50.7%</td>
<td>SE=64.8% SP=83%</td>
<td>SE=72.2% SP=76.9%</td>
</tr>
</tbody>
</table>

Rubaltelli et al. [28]. We also found that the elasticity ratios were different between benign and malignant nodule. On evaluating this method by ROC analysis we observed a best cut-off of >2.2 for differentiating malignant lesions (sensitivity: 85.2%, specificity: 97.1%, AUC:0.90). The elasticity ratio method had a better diagnostic performance than the Ueno elasticity scoring system (Figure 2). Consistent with our results, Abdelrahaman and his group reported a similar cut-off of >2.3 strain ratio predictive for malignancy [17].
enhancement. However, Nemec et al. observed no difference in the peak intensity of contrast agent between malignant and benign thyroid nodules. Similar to our results, Jiang et al. demonstrated no maximum intensity of contrast agent between malignant and benign nodules. However, there was significant difference in the TTP but a gross difference in the maximum intensity peak similar to normal thyroid tissue. An important indicator predicting benign nodules was ring enhancement of contrast due to a complete capsule around the nodule. Consistent with the results of our study, Ma et al., Zhang et al., and Jiang et al. observed ring enhancement as a predictor of benign thyroid nodules [20,31-33]

Majority of the follicular neoplasms 19/25 (76%) which were proved benign on histopathology demonstrated homogenous or ring with hyperrechoic or isoechoic enhancement and early or same time of arrival of contrast with respect to normal parenchyma. Follicular adenomas have richer, regular, capsular and rapid early inflow of blood supply when compared to normal thyroid tissue. On the contrary, strikingly, majority of the follicular neoplasms proved malignant (8/10; 80%) on postoperative HPE showed heterogeneous, hypoechoic and delayed arrival of contrast. Though, the latter FN’s have rich blood supply, tumour infiltration and proliferation may have caused destruction of large number of blood vessels leading to reduced and irregular blood flow [32,33]. Follicular neoplasm may probably best be differentiated by CEUSG.

Nodular goitre is characterised by homogenous, hyper/isoechoic enhancement with same or early arrival of contrast similar to normal thyroid tissue. The nodular goitre histologically has similar parenchyma with respect to normal tissue. Hence, the microvasculature pattern and regular distribution is similar to that of normal tissue [32,33]. In our study, 4 nodular goitres showed either no enhancement or hypoechoic enhancement of contrast. This pattern probably may be due to the destruction of microvasculature followed by haemorrhage.

Quantitative CEUSG using TIC analysis has gained importance in the recent times. The TIC reflects the vascularity in the thyroid and nodules. It has three phases: wash-in, peak enhancement and wash-out phase which constitute the area under the curve. The area under the curve reflects the duration in which the contrast remains in the thyroid nodule. Due to torturous and irregular vascularity of the malignant thyroid nodules, they present with delayed inflow of contrast and lower maximum intensity of the contrast with respect to normal parenchyma. On the other hand due to the abundant vascularity of nodular goitre and adenomas, there was a rapid arrival of contrast in the nodule and an increased maximum intensity peak similar to normal thyroid tissue.

We observed no difference in the TTP of contrast between benign and malignant nodules. However, there was significant difference in the maximum intensity of contrast agent between malignant and benign thyroid nodules. Similar to our results, Jiang et al. demonstrated no difference in the TTP but a gross difference in the maximum intensity of contrast agent between malignant and benign thyroid nodules. However, Nemec et al. observed no difference in the peak intensity enhancement [34].

The sensitivity specificity PPV, NPV and accuracy of qualitative CEUSG was 93.5%, 93.7%, 97.1%, 86.5% and 93.5%. Jiang et al. also observed a similar sensitivity specificity PPV, NPV and accuracy of 90%, 92%, 88%, 95% and 91%, respectively [33].

According to the results of the current study, a combined assessment of thyroid nodules with conjoint cUSG, elastographic and CEUSG techniques (AUC 0.99; 95% CI 0.96-1.00) rather than solely cUSG (AUC 0.97; 95% CI 0.94-0.994) or elastography and CEUSG alone (AUC 0.98; 95% CI 0.951-0.997) has proved valuable in the screening of thyroid nodules. On combining and ranking all parameters, heterogeneous contrast enhancement was observed to be the strongest indicator in predicting malignancy (OR:409, p-value-0.0002).

There was a limitation to our study. As our center was a tertiary reference centre, a large percentage of patients referred to us were previously investigated and had high suspicion of malignancy, which lead to patient selection bias resulting in higher rate of malignant lesions. Though this might affect the general prevalence of the nodules, it does not alter the strength of the results.

Conclusion
To conclude, although useful, no single ultrasonography parameter is a definitive predictor of malignancy. A conjoint analysis of specific features of thyroid nodules on cUSG, elastography and CEUSG will enhance the diagnostic value in the screening of thyroid nodules.

References