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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 histopathology 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:0.994.

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 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 Chammas 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 only 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 E1 was defined by drawing a circular ROI of 8 mm on normal thyroid tissue. E1 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 (E1) (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 ultrasound 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

Variables	Benign Nodules n=65	Malignant nodules N=108	p-value	Sensitivity	Specificity	PPV	NPV	Accuracy
Calcifications								
Microcalcification	8	70	<0.0001	64.8%	86.3%	88.6%	60%	70.3%
Macro/no calcifications	57	38						
Component								
Solid	31	77	0.003	71.3%	52.3%	71%	52.3%	77.1%
Solid+cystic/cystic	34	31						
Echogenity								
Isoechoic/hyerechois	33	2	<0.0001	98.1%	50.7%	76.8%	94.2%	80.3%
Hypoechoic	32	106						
Halo								
Complete	38	7	<0.0001	93.5%	58.4%	78.9%	84.4%	38.5%
Partial or no halo	27	101						
Margins								
Regular	54	38	<0.0001	64.8%	83%	86.4%	58.7%	70.3%
Irregular	11	70						
Colour Doppler								
Type -I/II	50	30	<0.0001	72.2%	76.9%	83.8%	62.5%	78.2%
Type -III	15	78						
Power Doppler								
Type-I/II/III	59	23	<0.0001	78.7%	90.2%	93.4%	71.9%	85.3%
Type -IV/V	6	85						
Ring enhancement								
Present	46	5						
Absent	19	103	<0.0001	95.3%	70.7%	84.4%	90.2%	86.1%
Arrival of contrast								
Early/same time	58	27	<0.0001	75%	89.2%	92%	68.2%	81.3%
Delayed	7	81						
Type of contrast enhancement								
Iso/hyper/no enhancement	63	19	<0.0001	82.4%	96.9%	97.8%	76.8%	89.3%
Hypo	2	89						
Distribution of contrast enhancement								
Homogenous	60	8	<0.0001	92.5%	93.2%	95.2%	88.2%	92.4%
Heterogeneous	5	100						
UENO Elasticity score								
Score 1/2/3	48	8	<0.0001	92.5%	73.8%	85.4%	85.7%	85.5%
Score 4/5	17	100						
Elasticity Index (mean ± SD)	1.96 ± 0.98	6.52 ± 1.32	<0.0001					
Elasticity ratio(mean ± SD)	1.388 ± 1.3	5.19 ± 2.6	<0.0001					
Time to peak (mean ± SD)	17.77 ± 6.6	19.21 ± 1.3	0.323					
Maximum intensity (Imax) (mean ± SD)	54 ± 5.4	42 ± 4.8	<0.0001					

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.

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 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 (Imax).

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 lymphocytic thyroiditis (BC:II), 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 rate were 41.5% (22/53). Among them, the malignancy rate in follicular neoplasm and suspicious cytology were 28.57% (10/35) and 66.6% (12/18) respectively. Figure 1 describes a flow chart regarding the details of FNAC, surgery and histopathology results

Histopathology results

Hundred and sixty one out of 173 thyroid nodules were operated at our hospital. In postoperative histopathologic examination thyroid cancer was detected in 108 nodules and 65 cases were reported as benign. The histopathological malignancy rate was 62.4% (108/173) Figure 1.

Conventional ultrasonography

The comparison of various characteristics on conventional ultrasound between benign and malignant nodules is described in Table 1. Univariate analysis showed Type-IV/V power Doppler patterns, microcalcifications and irregular margins as highly specific predictors of malignancy. On power Doppler, 59 of 65 benign nodules had Type-I-III vascularisation patterns and 85/108 malignant nodules had Type-IV/V vascularisation patterns. Among the 23 of 108 malignant nodules with Type-I-III flow patterns, majority were follicular neoplasms and suspicious for malignancy on FNAC 9/23 (39.1%). On multivariate logistic regression between conventional ultrasound parameters, Type-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, hypoechoic 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 hypoechoic 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, hypoechoic 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

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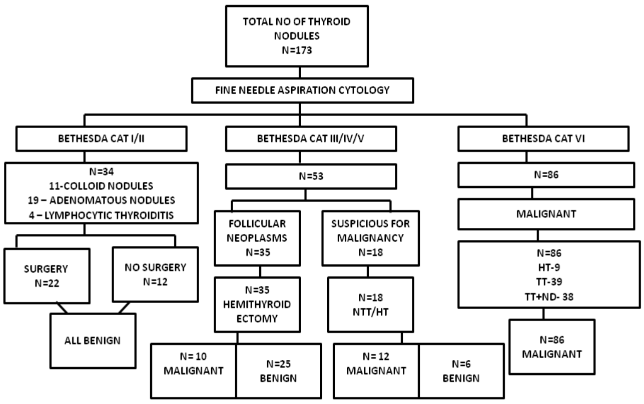

Figure 1: Detailed results of fine needle aspiration cytology, surgery and histopathology in all thyroid nodules.

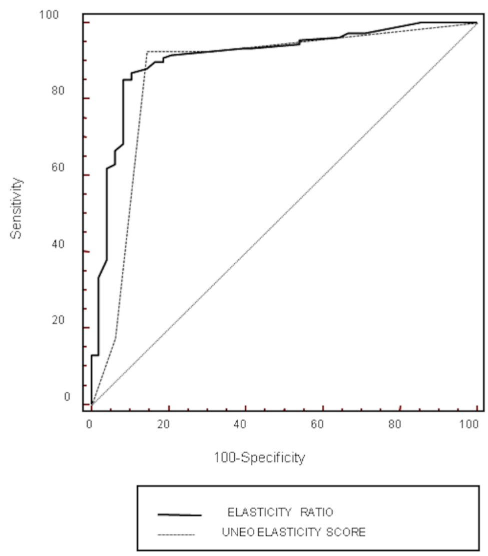

Figure 2: Receiver operating characteristic curves for elastography in differentiating malignant from benign lesions by elasticity ratio (solid line), cut-off >2.2, AUC: 0.90 and Ueno elasticity score (dotted line), cut-off >3, AUC: 0.86.

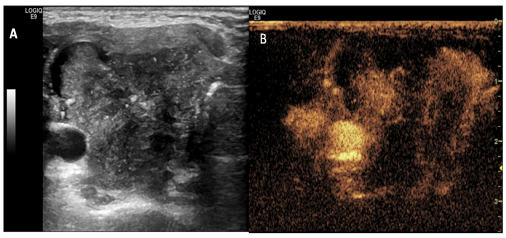

Figure 3: In a 18-year-old female with a palpable nodule in the right lobe of thyroid, histopathologically proven papillary carcinoma, gray-scale USG showed a hypoechoic mass with irregular margins, calcifications and absence of halo (A). CEUSG showed heterogeneous, hypoechoic, contrast enhancement (B).

93.8%, 95.3%, 97.2%, 89.8%, and 94.2%, respectively with AUC:0.98 (95% CI:0.951-0.997). The TICs of all nodules were analysed. The TICs of malignant nodules were characterised by lower peak signal intensity value compared to normal thyroid parenchyma (Figure 6A and 6B).

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

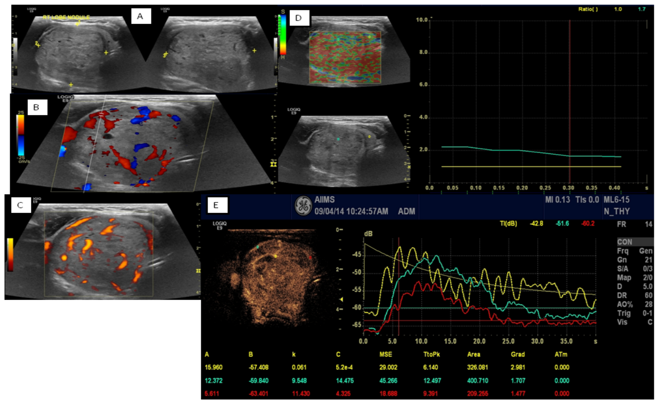

Figure 4: In a 52-year-old-female with a palpable nodule in the right lobe of thyroid, histopathology proved adenomatous goitre. (A) Gray-scale USG showed a isoechoic nodule with regular margins, presence of halo and type-III vascularization on both colour doppler and power doppler (B, C).On elastography the entire nodule represents mosaic pattern with Ueno elasticity score of 2 and elasticity ratio of 1 (D). CEUSG showed homogenous, hyperechoic, ring contrast enhancement (E). On TIC analysis (E) the yellow and green curve represents the lesion and the red curve represents the normal thyroid tissue. There is a difference in the maximum intensity value between the nodule and normal parenchyma.

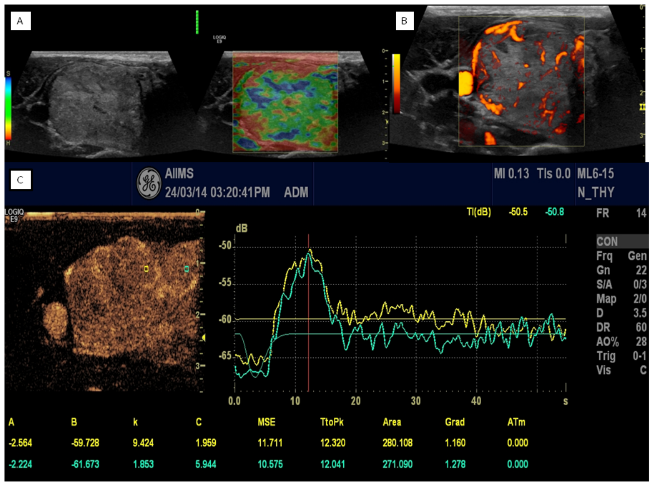

Figure 5: In a 17-year-old-male with a palpable nodule in the right lobe of thyroid, FNAC findings were suggestive of follicular neoplasm and histopathology proved papillary carcinoma thyroid. Gray-scale USG showed a isoechoic nodule (A) and Type-II blood flow pattern on power (B).On elastography the entire nodule represented mosaic pattern with Ueno elasticity score of 2 and elasticity ratio of 0.2 (A). CEUSG showed homogenous, isoechoic, ring contrast enhancement (C). On TIC analysis, green curve represents normal thyroid tissue and yellow curve represents thyroid nodule. There is no difference in the maximum intensity value and the time-to-peak between the nodule and normal parenchyma (C).

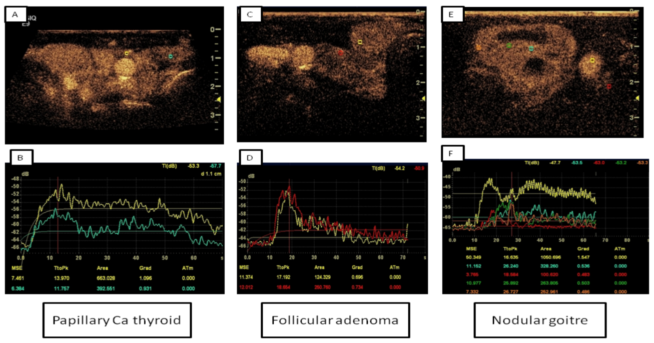

Figure 6: Comparison of contrast enhanced TIC curves in different thyroid nodules. (A,B) contrast enhanced image and TIC of papillary thyroid carcinoma nodule: the green curve represents the lesion and the yellow curve represents the normal thyroid tissue. (C,D) depict contrast enhanced image and TIC 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.

contrast enhancement, followed by Type-IV/V power Doppler flow patterns, absence of ring enhancement and elasticity ratio >2.2 with the largest AUC: 0.994; 95%; CI: 0.96-1.000 (Table 3).

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 and 61.8% histologically. Kim et al. reported a high malignancy rate of 20.8% cytologically [18]. On the other hand, Lin et al. [19] and Gul et al. [8] reported a malignancy rate of 3.4% and 3.3% cytologically and 3.95% and 7.6% histologically. The increased malignancy rate and the large variation were due to the differences in the patient selection, prevalence of >1 malignant nodule per patient in certain group of patients, type of FNAC procedure and reference of highly suspicious nodules to our clinic as ours is a tertiary super speciality setup. Of the 35 cases diagnosed as follicular neoplasm on cytology, the malignancy rate was 28.7% (10/35) on histopathology. These finding were in agreement with the Bethesda system for reporting thyroid cytopathology which reports malignancy rates of follicular neoplasms ranging from 15-30% [15]. Similarly, the malignancy rates of suspicious thyroid nodules (12/18; 66.6%) were in consonance with Bethesda system for reporting thyroid cytopathology [15].

Numerous studies have reported several gray-scale ultrasound parameters associated with thyroid cancer (Table 4) [7-9,20-25]. In the current study, the strongest conventional feature predictive for malignancy was Type-IV/V power Doppler pattern with sensitivity 78.7%, specificity 90.2%, PPV 93.4%, NPV, 71.7% and accuracy 85.3%, followed by microcalcifications and irregular margins. In concordance with our results, Chammas et al. reported power Doppler to facilitate screening of thyroid nodules with high risk of malignancy with an 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 hypoechogenicity 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 vascularisation 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, Shuzen 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.

The area under the curve for diagnosing malignant nodule by Ueno elasticity scoring system was 0.86. The results agree with Xing [29] and

VARIABLES	SE	p-value	OR	95% CI
Heterogeneous Contrast enhancement	1.330	0.0014	70.96	5.230-962.38
Hypoechoic contrast enhancement pattern	4.407	0.0007	82.08	6.474-1040
Ring enhancement of contract	1.591	0.0154	47.30	2.090-1070
Elasticity ratio	1.386	0.0082	39.07	2.582-591.30
SE: Standard Error; OR: Odds Ratio; 95% CI: 95% Confidence Interval.				

Table 2: Odds ratio of most predictive features for malignancy among contrast enhanced ultrasonography and elastography.

VARIABLES	SE	p-value	OR	95% CI
Heterogeneous Contrast enhancement	1.599	0.0002	409.31	17.795-9414
Type-IV/V power Doppler blood flow pattern	1.495	0.0002	283.17	15.104-5308
Ring enhancement of contract	1.5454	0.027	29.892	1.445-618.14
Elasticity ratio	1.43	0.0088	43.00	2.578-717.27
SE: Standard Error; OR: Odds Ratio; 95% CI: 95% Confidence Interval.				

Table 3: Odds ratio of most predictive features for malignancy among conventional ultrasonography, elastography and contrast enhanced ultrasonography features.

Author	N	Microcalcifications	Halo	Echogenicity	Margins	Blood flow patterns
Rago et al. (7)	92	SE=64% SP=72%	SE=61% SP=82%	SE=81% SP=62%	SE= -- SP=	SE=6.4% SP=97%
Gul et al. (8)	3404	SE=85.7% SP=98.2%	SE=98% SP=30.6%	SE=86% SP=67%	SE=90.2% SP=87.3%	SE=-- SP=
Sipo et al. (9)	-	SE=42.3% SP=91.2%	SE=63.6% SP=61.2%	SE=53% SP=73.2%	SE=57.7% SP=85.1%	SE=77.2% SP=79.3%
Ma et al. (20)	172	SE=66% SP=94.9%	SE=95.7% SP=51.3%	SE=77.7% SP=79.5%	SE=-- SP=	SE=-- SP=
Asteria et al. (21)	87	SE=59% SP=84%	SE=100% SP=14%	SE=65% SP=81%	SE=76% SP=78%	SE=-- SP=
Wang et al. (22)	51	SE=66% SP=75%	SE=-- SP=	SE= SP=	SE=57% SP=42%	SE=78% SP=47%
Rago et al. (23)	-	SE=59% SP=86 – 90%	SE= SP=-	SE=27-87 % SP=74-94%	SE=17-78% SP=39-85%	SE=54-74% SP=79-81%
Hong et al. (24)	145	SE=55% SP=94%	SE= SP=-	SE=86% SP=71%	SE=92% SP=84%	SE=35% SP=58%
Vito et al. (25)	97	SE=27% SP=78%	SE= SP=	SE=57% SP=72%	SE=62% SP=93%	SE=54% SP=28%
Present study	173	SE=64.8% SP=86.3%	SE=93.5% SP=58.5%	SE=98.1% SP=50.7%	SE=64.8% SP=83%	SE=72.2% SP=76.9%

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

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 clustered microcalcifications within the nodule. However, the optimal strain ratio cut-off values vary among studies. Unlike our results, Xing [29] and his colleagues reported a strain ratio of 3.79 as the cut-off, Kagoya et al. [30], reported >1.5 as the cut-off and Ning et al. stated 4.2 as the cut-off for predicting malignancy. This variation may be due to difference in the methodology of calculating strain ratios, and difference in the pathology of the nodules included in the study. 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.

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 number of vessels, distribution and structure and hence follow different patterns of contrast enhancement. In the current study qualitative analysis of contrast enhancement was done by the type of distribution of contrast, echogenicity of uptake of contrast and arrival of contrast with respect to normal parenchyma. Quantitative analysis included time to peak, maximum intensity of contrast.

Among all cUSG, elastography and CEUSG parameters, the most important factor predicting the malignancy of thyroid nodules was heterogeneous enhancement of contrast with an OR of 409.31 (95%

CI: 17.79-9414.59), 92.5% sensitivity, 93.2% specificity, and 92.4% accuracy (Table 3). The uniform regular distribution of blood vessels in benign nodules reflects the homogenous and isoechoic enhancement of contrast with respect to normal thyroid tissue. On the other hand, malignant nodules present with microcalcifications, necrosis and fibrosis which affect the tumour angiogenesis. Hence, there is decreased microvascular density leading to decreased blood supply reflecting the hypoechoic enhancement of contrast agent in the malignant tissue compared 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 hyperechoic 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 FNs' 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 vascularisation 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%, 93% 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:0.942-0.994) 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 a 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

1. Vander JB, Gaston EA, Dawber TR (1968) The significance of nontoxic thyroid nodules. Final report of a 15-year study of the incidence of thyroid malignancy. Ann Intern Med 69: 537-540.
2. Tunbridge WM, Evered DC, Hall R, Appleton D, Brewis M, et al. (1977) The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol (Oxf) 7: 481-493.
3. Davies L, Welch HG (2014) Current thyroid cancer trends in the United States. JAMA Otolaryngol Head Neck Surgn 140: 317-322.
4. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, et al. (2016) American Thyroid Association Management guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 26: 1-134.
5. Danese D, Sciacchitano S, Farsetti A, Andreoli M, Pontecorvi A (1998) Diagnostic accuracy of conventional versus sonography-guided fine-needle aspiration biopsy of thyroid nodules. Thyroid 8: 15-21.
6. Carmeci C, Jeffrey RB, McDougall IR, Nowels KW, Weigel RJ (1998) Ultrasound-guided fine-needle aspiration biopsy of thyroid masses. Thyroid 8: 283-289.
7. Rago T, Santini F, Scutari M, Pinchera A, Vitti P (2007) Elastography: new developments in ultrasound for predicting malignancy in thyroid nodules. J Clin Endocrinol Metab 92: 2917-2922.
8. Gul K, Ersoy R, Dirikoc A, Korukluoglu B, Ersoy PE, et al. (2009) Ultrasonographic evaluation of thyroid nodules: comparison of ultrasonographic, cytological, and histopathological findings. Endocrine 36: 464-472.
9. Sipos JA (2009) Advances in ultrasound for the diagnosis and management of thyroid cancer. Thyroid 19: 1363-1372.
10. Molinari F, Mantovani A, Deandrea M, Limone P, Garberoglio R, et al. (2010) Characterization of single thyroid nodules by contrast-enhanced 3-D ultrasound. Ultrasound Med Biol 36: 1616-1625.
11. Ueno E, Ito A Diagnosis of breast cancer by elasticity imaging. Eizo Joho Medical 36: 2-6.
12. Imaizumi A, Sasaki Y, Sakamoto J, Kamio T, Nishikawa K, et al. (2014) Effects of compression force on elasticity index and elasticity ratio in ultrasound elastography. Radiol 43: 1-6.
13. Ning CP, Jiang SQ, Zhang T, Sun LT, Liu YJ, et al. (2012) The value of strain ratio in differential diagnosis of thyroid solid nodules. Eur J Radiol 81: 286-291.
14. Chammas MC, Gerhard R, de Oliveira IR, Widman A, de Barros N, et al. (2005) Thyroid nodules: evaluation with power Doppler and duplex ultrasound. Otolaryngol Head Neck Surg 132: 874-882.
15. Cibas ES, Ali SZ (2009) The Bethesda System for Reporting Thyroid Cytopathology. Thyroid 19: 1159-1165.

16. Hedinger C, Williams ED, Sobin LH (1989) The WHO histological classification of thyroid tumors: a commentary on the second edition. Cancer 63: 908-911.

17. Abdelrahman SF, Ali FH, Khalil ME, Masry MRL (2015) Ultrasound elastography in the diagnostic evaluation of indeterminate thyroid nodules. The Egyptian journal of Radiology and nuclear medicine 46: 639-642.

18. Kim DL, Song KH, Kim SK (2008) High prevalence of carcinoma in ultrasonography-guided fine needle aspiration cytology of thyroid nodules. Endocr J 55: 135-142.

19. Lin JD, Chao TC, Huang BY, Chen ST, Chang HY, et al. (2005) Thyroid cancer in the thyroid nodules evaluated by ultrasonography and fine-needle aspiration cytology. Thyroid 15: 708-717.

20. Ma JJ, Ding H, Xu BH, Xu C, Song LJ, et al. (2014) Diagnostic performances of various gray-scale, color , and contrast-enhanced ultrasonography findings in predicting malignant thyroid nodules. Thyroid 24: 355-365.

21. Asteria C, Giovanardi A, Pizzocaro A, Cozzaglio L, Morabito A, et al. (2008) US-elastography in the differential diagnosis of benign and malignant thyroid nodules. Thyroid 18: 523-531.

22. Wang Y, Dan HJ, Dan HY, Li T, Hu B (2010) Differential diagnosis of small single thyroid nodules using real time ultrasound elastography. J Int med Res 38: 466-472.

23. Rago T, Vitti P (2009) Potential value of elastosonography in the diagnosis malignancy in in thyroid nodules. QJ nucl Med Mol imaging 53: 455-464.

24. Hong Y, Xueming L, Zhiyu L, Zhang X, Chen M, et al. (2009) Real-time ultrasound elastography in the differential diagnosis of benign and malignant thyroid nodules. *J Ultrasound Med* 28: 861-867.

25. Cantisani V, D'Andrea V, Biancari F, Medvedyeva O, Di Segni M, et al. (2012) Prospective evaluation of multiparametric ultrasound and quantitative elastosonography in the differential diagnosis of benign and malignant thyroid nodules: preliminary experience. Eur J Radiol 81: 2678-2683.

26. Rago T, Vitti P, Chiovato L, Mazzeo S, De Liperi A, et al. (1998) Role of conventional ultrasonography and color flow-sonography in predicting malignancy in 'cold' thyroid nodules. Eur J Endocrinol 138: 41-46.

27. Shuzhen C (2012) Comparison analysis between conventional ultrasonography and ultrasound elastography of thyroid nodules. Eur J of Radiol 81: 1806-1811.

28. Rubaltelli L, Corradin S, Dorigo A, Stabilito M, Tregnaghi A, et al. (2009) Differential diagnosis of benign and malignant thyroid nodules at elastosonography. Ultraschall Med 30: 175-179.

29. Xing P, Wu L, Zhang C, Li S, Liu C, et al. (2011) Differentiation of benign from malignant thyroid lesions: calculation of the strain ratio on thyroid sonoelastography. J Ultrasound Med 30: 663-669.

30. Kagoya R, Monobe H, Tojima H (2010) Utility of elastography for differential diagnosis of benign and malignant thyroid nodules. Otolaryngol Head Neck Surg 143: 230-234.

31. Zhang B, Jiang YX, Liu JB, Yang M, Dai Q, et al. (2010) Utility of Contrast-Enhanced Ultrasound for Evaluation of Thyroid Nodules. Thyroid 20: 57-67.

32. Jiang J, Huang L, Zhang H, Ma W, Shang X, et al. (2014) Contrast-Enhanced Sonography of Thyroid Nodules. J Clin Ultrasound 243: 153-156.

33. Jiang J, Shang Xu, Wang H, Yong-Bo X, Gao Y, et al. (2015) Diagnostic value of contrast-enhanced ultrasound in thyroid nodules with calcification. Kaohsuing Journal of Medical Sciences 31: 138-144.

34. Nemec U, Nemec SF, Novotny C, Michael Weber M, Czerny C, et al. (2012) Quantitative evaluation of contrast-enhanced ultrasound after intravenous administration of a microbubble contrast agent for differentiation of benign and malignant thyroid nodules: assessment of diagnostic accuracy. Eur Radiol 22: 1357-1365.