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Original Article

Radiology Section

Role of Colour Doppler for Assessment of Malignancy in Solid Breast Masses: A Prospective Study

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ABSTRACT

Introduction: Mammography and ultrasound are the commonly used modalities for radiological evaluation of breast masses and assessment of aggressive features that point towards malignancy. Colour Doppler provides vital information about the vascularity of solid breast masses. Neoangiogenetic vascular architecture of the solid breast lesions can be depicted reliably on colour Doppler, some differentiating features of benign and malignant breast masses on colour Doppler can also provide additional critical information for the assessment of malignancy.

Aim: To assess the role of colour Doppler ultrasound in differentiating benign and malignant breast masses and to ascertain the predictive value of colour Doppler in predicting malignancy grade in FNAC proven malignant breast masses.

Materials and Methods: We prospectively studied features of Doppler ultrasound on 173 treatment-naïve solid breast masses in 148 patients diagnosed in our institution. Doppler ultrasound findings of all these masses were evaluated using colour, power and spectral Doppler. Cytological analysis of all the masses was done by Fine Needle Aspiration Cytology (FNAC). Various colour Doppler parameters were correlated with findings on FNAC. Total 52 malignancies of the breast were detected and each malignant mass was given a grade (1, 2 or 3) on FNAC as per Robinson's cytological grading system. Statistical correlation was made between colour Doppler findings and cytological results.

Results: In our study we found colour Doppler characteristics of hypervascularity, tortuous arteries with irregular caliber, presence of a penetrating artery and a central pattern of distribution of vessels as signs significantly associated with malignancy. Mean RI of malignant masses was noted to be higher than benign masses, however no statistically significant correlation was observed between RI values and the grade of malignancy.

Conclusion: Colour Doppler and power Doppler sonography are tools which provide useful information about the vascularity of a mass, hence these should be used routinely in the evaluation of all breast masses. Color Doppler does add vital supplementary information in the characterisation of breast masses on ultrasound, however in isolation, it is still limited in the specific differentiation of benign and malignant masses.

Keywords: Breast cancer, Hypervascularity, Neoangiogenesis, Penetrating vessel

INTRODUCTION

Breast cancer is the leading cause of deaths (15%) among females, with over 1 million new cases in the world each year [1]. Ultrasound is a safe, non-invasive and radiation free adjunct to mammography for the evaluation of palpable breast masses. It is also used as a primary screening tool for breast cancer in women who are pregnant, young women with high risk for breast cancer and who have increased breast density on mammogram [2]. Some recent studies have investigated the role of colour Doppler ultrasound for characterisation of breast lesions [3-5].

MATERIAL AND METHODS

We prospectively studied features of Doppler ultrasound on 173 treatment-naïve solid breast masses in 148 patients (145 female and 3 males) diagnosed in our institution between the period of March 2014 to June 2016. The study was conducted in ESI Medical College and PGIMSR, K K Nagar, Chennai, India. The study was approved by the Institutional Review Board and an informed consent was obtained from all patients for participation in this study. We assessed the breast masses in the Department of Radiodiagnosis using colour, spectral and power Doppler as an adjunct to ultrasound. The age range of the patients was from 15 years to 78 years. Ultrasound

was used to assess if a palpable mass was solid or cystic. Solid masses were optimally assessed by colour and spectral Doppler and subsequently evaluated by a pathologist using FNAC technique. Out of 173 solid masses, 52 were proven to be malignant on cytology, while the rest were benign.

Inclusion criteria: Inclusion criteria for the study was any patient with solid space occupying lesion in the breast on ultrasound and had colour Doppler examination and subsequently had FNAC.

Exclusion criteria: Cases of primarily cystic breast masses, patients with prior surgery for a breast mass and patients with inconclusive findings on cytology, such as inadequate tissue sample. Patients who did not get FNAC and patients who were lost to follow-up were excluded from the study. We also excluded breast lesions on which serial follow-up ultrasound was recommended and we did not have pathology results.

Methodology

After obtaining informed consent, we assessed the patients with colour Doppler and ultrasound. This examination was performed on a Philips ultrasound machine unit (HD7/ Clearvue 650) using a linear, high frequency probe (3-12MHz). A curvilinear transducer (2-5 MHz) was used when needed. Subsequently, the patient underwent ultrasound guided FNAC within a week after the initial ultrasound examination. The cytological study was done by a pathologist in our institution. Each solid breast mass was evaluated by one of the two trained radiologists with experience of ten or more years. Images in various planes were taken during real time imaging and stored in a JPEG format. For each of the 173 solid masses, the following criteria were assessed by colour Doppler:

1. Presence or absence of vascularity using colour and power Doppler.
2. The number of arteries within the mass: presence of 1-2 arteries were categorised as hypovascular, presence of three or more arteries within the mass were termed as hypervascular masses [4].
3. The distribution (central or peripheral, mixed, capsular) of vessels [3].
4. The morphology of vessels, whether regular or irregular and/or tortuous [3].
5. The arrangement of vessels, whether smooth with regular branching or chaotic [3].
6. Presence/ absence of penetrating arteries, i.e. a vessel entering into the mass perpendicular to it, without passing through the capsular vessels [3,5].
7. Spectral analysis of the arteries using Peak Systolic Velocity (PSV) and Resistivity Index (RI).

FNAC of the breast mass was performed to reach the final diagnosis. The samples were reviewed in detail by a consultant pathologist who was not aware of Doppler sonographic data. For each malignant mass, cytological grading was made by the pathologist using Robinson's cytological grading.

In our study we evaluated whether a contribution of colour and spectral Doppler can be used to provide complementary information to gray scale ultrasound findings of malignant masses to predict the grade of malignancy.

Technical Considerations: High frequency linear array transducers (7-13MHz) are ideal for Doppler evaluation of breast masses enabling us to evaluate signals in small vessels less than 0.1 mm in diameter with low velocity flow [3,5]. For Doppler evaluation of breast masses, we optimised the following machine settings for detecting vascularization in small diameter vessels: maximum gain settings (85-90%), PRF between 7 to 10 kHZ, wall filter as low as possible (50 to 100Hz max), appropriate algorithm to remove motion artifacts with medium persistence. Doppler box was taken without angulation. The pressure on the breasts was kept to a minimum to avoid the compression of blood vessels, thus enabling us to detect low blood flow. We used power Doppler in all cases that failed to detect colour flow on colour Doppler mode, since it is doppler angle independent and hence, not subjected to aliasing, thereby providing better detection of slow flow.

STATISTICAL ANALYSIS

In our study, we used descriptive statistics for quantitative data presented by N, mean, Standard deviation and range. For qualitative data, frequency count n and percentage were displayed in a tabular manner. Appropriate statistical tests were applied to analyse the data to find an association between two attributes. Chi square test has been used by using statistical software SPSS (version 21.0). Sample size is based on the proportion for one group to find the confidence interval-estimating single proportion. In previous study, the prevalence rate of breast cancer was 15% [1], taking desired confidence rate as 95% with absolute precision 6, the required sample size was 136. After consideration of dropouts, it was decided to take up to 150 subjects.The sample size calculation was done by using nMaster software (version 2.0).

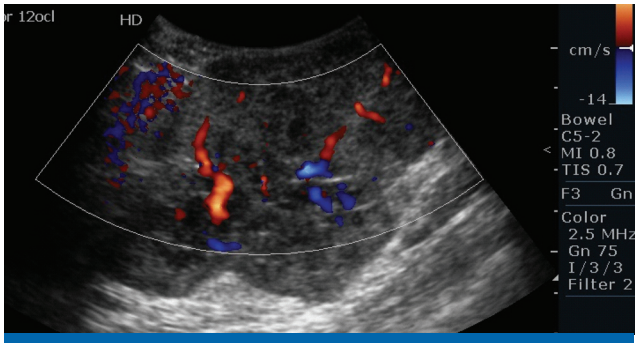
RESULTS

Colour Doppler examination and FNAC were performed on 173 solid breast masses in 148 patients (145 females, 3 males). The patients ranged in age from 15 to 78 years. The mean age for the benign lesions was 35 years while for malignant lesions it was 49 years. Of all the masses (173), FNAC diagnosed 121 masses as benign lesions and 52 masses as malignant. As expected, benign group of pathologies were seen in an average younger age group. The most common pathology encountered in benign category was fibroadenoma (68.6%) while the most common pathology in malignant lesions was invasive ductal carcinoma (90.4%) [Table/Fig-1]. Doppler characteristics of all the lesions were evaluated and compared

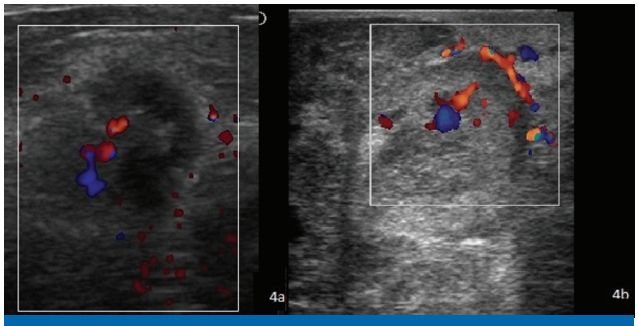
in benign and malignant groups. Vascularity was detected in 46 (38%) of 121 benign masses and in 43 (82.7%) of 52 malignant masses, using colour and power Doppler [Table/Fig-2]. Hypervascularisation was seen in 77% of all malignant breast masses, as compared to the surrounding tissue [Table/Fig-3-6]. However, in the benign masses only 8.26% depicted hypervascularisation (fibroadenoma and phyllodes tumors). It was noted in our study that in colour Doppler evaluation of a solid breast mass, hypervascularity proved to be a reliable sign to predict the possibility of malignancy, as pointed out by various authors previously [Table/Fig-3] [4-6]. Amongst the 46 benign lesions that revealed vascularity, only 10 benign lesions show evidence of hypervascularity [Table/Fig-6]. Rest of the vascular benign lesions showed presence of capsular vessels or 1-2 arteries distributed in the peripheral portion of the mass [Table/Fig-7]. In malignant lesions, arteries were arranged in the central part of the lesion with/without vessels in the periphery [Table/Fig-3,4]. The vessels in benign masses were generally of regular caliber with regular branching. Out of 43 malignant lesions that show vascularity on colour Doppler, 31(72%) masses depicted tortuous arteries with irregular caliber, arranged in a chaotic pattern [Table/Fig-4,5,8]. About 62% of the malignant lesions had the presence of a penetrating artery [Table/Fig-9]. Spectral analysis was done on all vascular masses and PSV and RI noted. The mean values of RI in benign and malignant lesions were 0.63 $\pm$ 0.08 and 0.80 $\pm$ 0.16 respectively [Table/Fig-2]. The 43 vascular malignant lesions had a mean RI value of 0.80 with SD of 0.16 (range, 0.50–1.02) [Table/Fig-2]. Based on threshold value of 0.7, sensitivity and specificity of RI values in differentiating benign and malignant masses were 88% and 57% respectively. Previous studies have suggested that reversal or absence of diastolic flow on spectral Doppler makes it a highly probably finding of malignancy with a specificity of 99% and positive predictive value of 97% [4,7]. In our study, out of 43 vascular

Diagnosis by FNAC	Number of Cases
Normal breast fat nodule	6
Fibroadenoma	83
Fibrocystic changes	10
Intramammary lymph node	9
Papilloma	5
Gynecomastia	2
Phyllodes tumour	6
Invasive ductal carcinoma	47
Ductal carcinoma in-situ	2
Lobular breast carcinoma	1
Metaplastic tumour	2
Total	173

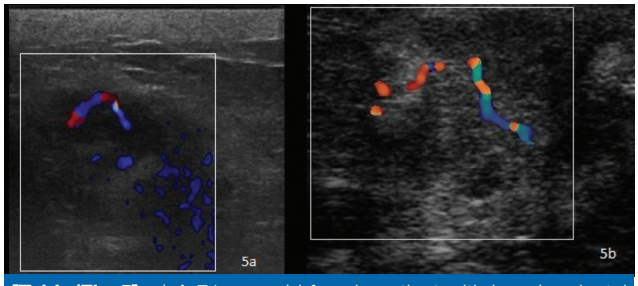
[Table/Fig-1]: Breast masses and their cytological diagnoses.



[Table/Fig-3]: A 54-year-old female patient with invasive ductal carcinoma in the breast showing presence of hypervascularisation on colour Doppler ultrasound.



[Table/Fig-4]: a) A 38-year-old female patient with invasive ductal carcinoma of the breast showing presence of hyper vascularity on colour Doppler. b) A 54-year-old female patient with invasive ductal carcinoma in the breast showing presence of hypervascularisation on colour Doppler ultrasound with vessels in a chaotic distribution.



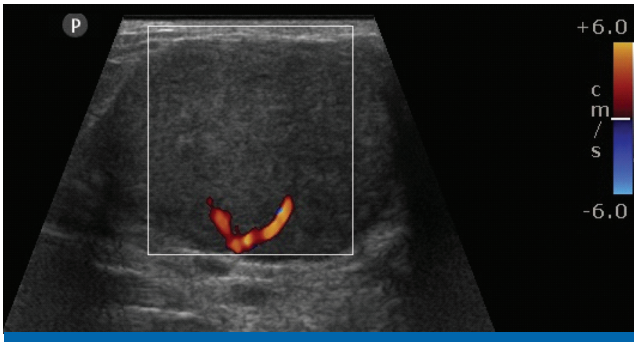
[Table/Fig-5]: a) A 71-year-old female patient with invasive ductal carcinoma of the breast showing vessel with irregular caliber and arranged in a tortuous manner; b) A 51-year-old female patient with invasive ductal carcinoma in the breast showing a tortuous vessel with irregular caliber on colour Doppler.

Pathology	Vascular on Color Doppler	Avascular on Colour Doppler	Total	Chi-square Test, df	p-value	RI Mean + SD	Range of RI
No (%) of Benign Lesions	46 (38%)	75 (62%)	121	29.06, 1	< 0.001	0.63 $\pm$ 0. 08	0.53 - 0.75
No (%) of Malignant Lesions	43 (82.7%)	9 (17.3%)	52			0.80 $\pm$ 0.16	0.50 - 1.02

[Table/Fig-2]: Comparison of colour and spectral Doppler findings in benign and malignant breast lesions.

Pathology	Hypervascular on Colour Doppler	Hypovascular on Colour Doppler	Avascular on Colour Doppler	Total	Chi Square Test, df	p-value
No. (%) of Benign Lesions	46 (38%)		75 (62%)	121	83.55, 2	<0.001
	10 (8.26%)	36 (29.74%)				
No. (%) of Malignant Lesions	43 (82.7%)		9 (17.3%)	52		
	40 (77%)	3 (5.7%)				

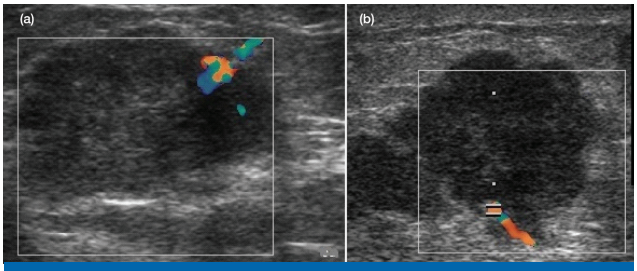
[Table/Fig-6]: Table/Fig-6: Degree of vascularity of benign and malignant masses on colour Doppler.



[Table/Fig-7]: A 29 year old female patient with fibroadenoma of the breast showing presence of vessels limited to the periphery on power Doppler.

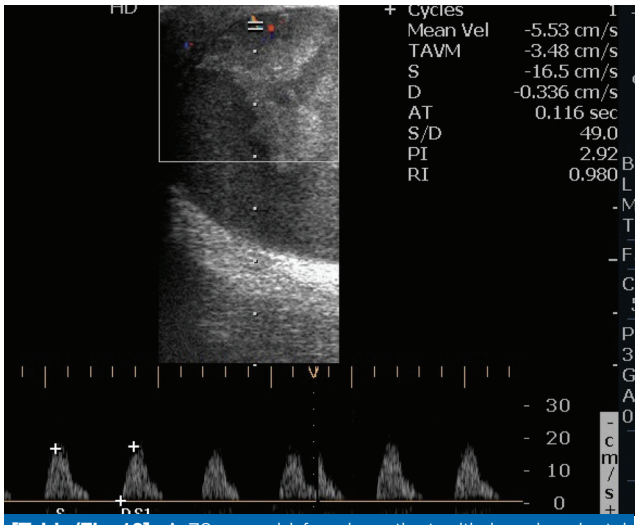
Morphology of Arteries on Colour Doppler	Benign Vascular Masses on Colour Doppler		Malignant Vascular Masses on Colour Doppler		Chi Square Test, df	p-value
	Number	%	Number	%		
Regular Caliber	42	91	12	28	37.44, 1	< 0.001
Tortuous and/ or Irregular Caliber	4	9	31	72		
Total	46		43			

[Table/Fig-8]: Morphology of arteries in breast masses that depicted vascularity on colour Doppler.

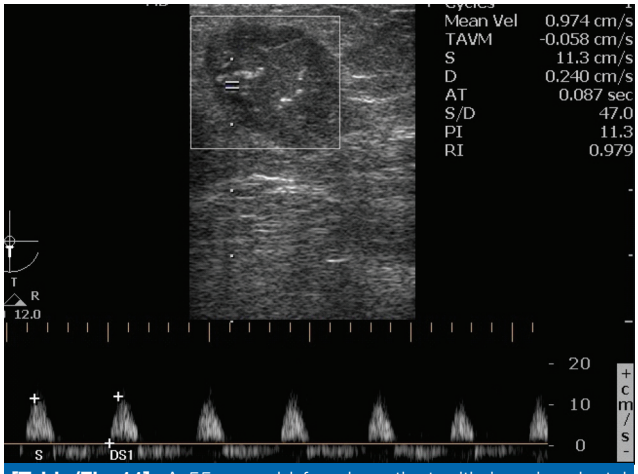


[Table/Fig-9]: a) A 62-year-old female patient with invasive ductal carcinoma of the breast showing the presence of penetrating artery on colour Doppler. b) A 48 year old female patient with invasiveductal carcinoma of the breast grade 3 showing the presence of penetrating artery. RI=0.98.

malignant masses only eight depicted the presence of loss or reversal of flow in diastole [Table/Fig-10,11]. This finding was not seen in any of the benign masses. Hence, absence or reversal of diastolic flow appears as an important signature



[Table/Fig-10]: A 78-year-old female patient with invasive ductal carcinoma in the breast grade 2. Spectral analysis reveals presence of absent flow in diastole.



[Table/Fig-11]: A 55-year-old female patient with invasive ductal carcinoma in the breast grade 3. Spectral analysis reveals presence of reversal of flow in diastole.

pattern of malignant masses. An interesting point to note in our study was that 7 of 8 malignancies depicting this sign were high grade on pathology (grade 2/3).

Since, only limited work has been done on establishing correlation between imaging features and biological behavior of tumours, we compared the Doppler features of all the malignant lesions with their grades on cytological analysis

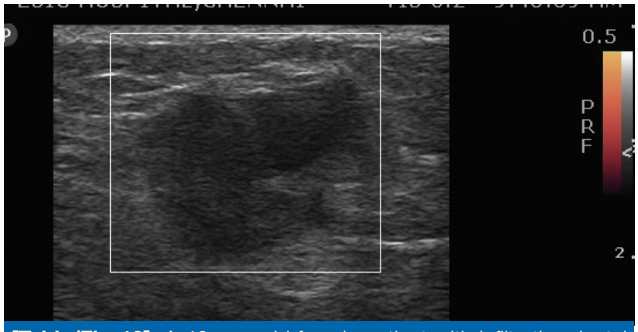
[8]. All the 52 malignant masses were divided into grades 1, 2 and 3 using Robinson's cytological grading [Table/Fig-12]. On FNAC, each malignant mass was assessed based on the following criteria, cellular dissociation, cell size, cell uniformity, nuclear membrane, nuclear chromatin and mitotic features to derive at a final grade of 1, 2 or 3. All the descriptors of colour Doppler in malignant masses were compared with their respective grades on FNAC. Due to overlap of various Doppler features in all the 3 grades of tumours, no statistically significant association was noted. As stated previously, eight cases of malignant carcinomas revealed a reversal/lack of diastolic flow on spectral Doppler, out of which seven malignancies were of grade 2 or grade 3 on cytological analysis [Table/Fig-10,11].

FNAC Grade	Number of Malignant Masses	Percentage (%)	Test	p-value	Decision
1	28	54%	one-sample Chi-square test	0.007	The Null hypothesis rejected
2	13	25%			
3	11	21%			
Total	52	100			

[Table/Fig-12]: Malignant masses with their cytological grading as per Robinson's cytological grading.
Null Hypothesis: The categories of FNAC occur with equal probabilities.

DISCUSSION

Colour Doppler is an adjunct tool to ultrasound imaging findings in differentiating malignant from benign masses of the breast [4,6,7]. In younger patients with dense breast tissue, ultrasound is more sensitive (95-99%) than mammography (85%) for the detection of breast cancer with relatively increased detection of early breast carcinomas of 22.5% found in supplemental breast ultrasound examination [2]. Colour Doppler is an easy to use tool present in most ultrasound machines and gives information about the vascularity without the use of contrast medium. Both benign and malignant masses in the breast can show increased vascularity, however, in malignant masses, the increased vascularity is caused by neoangiogenesis. The new vessels in malignancy are tortuous, non-hierarchical and disorganised with frequent stenosis, occlusions and arteriovenous shunts [4,7]. Tumour cells produce protein angiogenin, thus correlating with tumor growth and producing new vessels that are tortuous, of irregular caliber and thin walls with no smooth muscle [3,4]. Signs of benignity on colour Doppler are capsular blood vessels with straight or curved path, regular caliber and harmonious distribution [4]. A prior study by Horvath et al., found that 98.5% of masses exhibiting the finding of 'parallel artery and vein' were benign considering that an artery accompanied by its vein denotes a normal anatomical location [5]. Our colour Doppler analysis revealed that the majority of malignant masses were characterised by hypervascularity (77%) with peripheral and central vascularity, tortuous arrangement of vessels with varying caliber (72%) and presence of a penetrating artery (62%) [Table/Fig-6,8]. Although, hypervascularity of a breast lesion on colour Doppler increases the likelihood of malignancy, apparent avascularity on Doppler should not be interpreted as a sign of benignity [4,6]. In all 17.3% of malignant lesions in our study were avascular on power Doppler [Table/Fig-6,13]. This finding is attributed to very small caliber vessels within the mass. A mass with central vessels and lack of capsular vessels is suspicious for malignancy. Identification of penetrating vessels entering the mass directly, without passing through the capsular vessels, represent a finding highly suggestive of malignancy [4]. Previous studies have shown that a high RI increases the chances of malignancy in a breast mass, mainly related to presence of areas of stenosis, occlusions and arteriovenous shunts and lack of smooth muscle [4, 7]. However, studies have used different RI values as the cut-off points for differentiating benign and malignant breast masses. Lee et al., reported a threshold of RI of 0.78 in differentiating benign from malignant breast masses [9]. Choi et al., observed that in more than 80% of breast masses, RI exceeded 0.70 with a sensitivity of 80.9% and specificity of 89% [10]. In our study, we also found a higher RI value in malignant masses as compared to benign masses. Many authors have reported that the analysis of spectral Doppler criteria such as RI and PI values contribute less in differentiation between benign and malignant breast masses than colour Doppler [3,11,12]. This is because though malignancies are known to have a higher RI and wider range than benign lesions on spectral Doppler, there is a wide overlap of spectral parameters, hence RI cannot be relied upon in differentiating benign from malignant masses [4,5,10]. There is no standard cut off RI values to differentiate between the two, however, absence or reversal of diastole on spectral Doppler is a very specific Doppler sign for predicting malignancy in solid breast masses [4,7]. Disappearance or reversal of vascular flow represents extreme increase of peripheral resistance of tumoral vessels, due to previously mentioned phenomenon of stenosis and occlusions, typical of malignant vascularity [7]. In our study, we found a total of eight cases of malignant masses with reversed/absent diastolic flow. The disappearance or reversal of vascular flow in the diastole in malignancies is indicative of the presence of very high resistance flow. RI is 1 when the



[Table/Fig-13]: A 40-year-old female patient with infiltrative ductal carcinoma in the breast grade 2. Power Doppler reveals lack of vascularity on colour Doppler.

diastolic flow is absent and RI is >1 when the flow is reversed on diastole. This sign on spectral Doppler has high positive predictive value for detection of malignancies, as also the presence of a penetrating artery on power Doppler [7,13]. In our case, eight malignant masses depicted a lack/reversal of diastolic flow, out of which all but one mass proved out to be of high grade (2 or 3), indicating that reversal/lack of diastolic flow detection on spectral analysis of a suspicious breast mass is indicative of the aggressiveness of the lesion. The theory to this association is that the higher the resistance, the greater the shear forces induced on the tumour cells by the laminar flow, favoring the detachment of tumour cells [4,5]. However a larger size study could have allowed a better insight into the association between reversal/end of diastole and higher grades of malignancies. A study with larger sample size may be needed to prove a statistically significant correlation between the lack/reversal of diastolic flow and increased aggressiveness.

Ultrasound imaging additionally plays a crucial role in interventional procedures as FNAC, biopsy and preoperative localisation. However, ultrasound when added to mammography screening can increase the biopsy rates to 2.3-4.7% as against a 1-2% with only mammography screening [2]. This trend of increased biopsy rates due to the use of additional ultrasound can be optimised to an extent by the routine use of colour Doppler tool in all solid breast lesions, to aid in the differentiation between benign and malignant lesions [14]. With the use of the colour Doppler tool in masses where gray scale predictors suggest benignity, presence of 'benign' doppler signs reinforce the benign appearance of mass on ultrasound, possibly allowing for a reduction in BIRADS scoring (for example from BIRADS 4a to BIRADS 3) in a likely benign/indeterminate lesion, thus reducing unnecessary biopsies [15,16]. For such lesions, follow-up can be safely recommended with a higher degree of diagnostic confidence, compared to ultrasound alone. Similarly, a lesion with typical high grade features of malignancy on colour Doppler supports the need for urgent histological verification, thus aiding the referring surgeon in the management of these cases. Lesion evaluation under colour Doppler before performing biopsies under ultrasound guidance is recommended to limit the number of unnecessary biopsies and also to use vasoconstrictors with local anesthesia in case of hypervascular lesions to reduce the risk of bleeding.

LIMITATION

The sample size is relatively small. However, this study is aimed to decipher if results can lead us to conduct a bigger, multicenter study using Doppler as a major diagnostic tool for predicting the grade of aggressiveness of the tumor. Secondly, the final diagnosis came from samples collected by fine needle aspiration technique which means a limited sample as compared to a core needle biopsy technique. However, for the resource poor settings, FNAC is cheaper, less invasive and can sample different areas of the lesion compared with core needle biopsy. Thirdly, colour Doppler tool in ultrasound is operator- and equipment-dependent, exhibiting a wide inter-observer variability.

CONCLUSION

With recent developments in technique, colour Doppler ultrasound is now an important diagnostic tool for the evaluation of breast lesions. In our study we found hypervascularity, tortuous arteries with irregular caliber, presence of a penetrating artery and a central pattern of distribution of vessels as signs significantly associated with malignancy. No correlation was observed between various spectral Doppler parameters such as RI values and the grade of malignancy.

Our study re-emphasises the fact that hypervascularity itself, in the lack of other gray scale features, may not be an adequate predictor of malignancy and lack of vascularity cannot rule out a possibility of malignancy. However, if a lesion shows no aggressive features on ultrasound and has Doppler features of `benignity`, follow-up can be safely recommended, obviating the need for tissue sampling. In isolation however, color Doppler findings cannot be considered as a sole predictor of malignancy. We were unable to establish a relationship between vascularization and tumour grades. Though histopathology remains the gold standard for diagnosis of breast lesions, our study suggests that colour and spectral Doppler analysis be incorporated in the routine ultrasound evaluation of all solid breast masses.

ADVANCES IN KNOWLEDGE

- On spectral Doppler, lack of diastolic flow/reversal of diastole is a highly specific feature of malignancy.
- Lack of diastolic flow/reversal of diastole may be associated with high grade of malignancy, independent of other factors.
- Hypervascularity itself, in the lack of other gray scale features, may not be an adequate predictor of malignancy and lack of vascularity cannot rule out a possibility of malignancy.
- There is a general trend towards high RI values in malignant breast masses, however there is no correlation between RI values and increased aggressiveness of the malignant lesion.
- RI ≥ 1 is highly suggestive of malignancy in a breast mass, even in the absence of any other suspicious signs of malignancy in the breast mass.

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