

Original Research Article

Triple Test: A 'One Stop' Diagnostic Test in Evaluation of Breast Lesions and its Correlation with Histopathology

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Abstract: The aim of this study was to assess the role of Triple Test in preoperative diagnosis of palpable breast lesions and to evaluate performance characteristics of its components individually and combined, keeping histopathological examination as reference standard. A total of 120 cases of palpable breast lesions were studied over a period of five years from 2009 to 2014. These patients were subjected to detailed clinical breast evaluation, mammography and/or ultrasonography and fine needle aspiration cytology. Each component was categorized into benign, suspicious and malignant. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of each test were determined individually and as combined. Patients with age ranging 18-80 years were evaluated by Triple Test. The test concurred in 118 (102 benign and 16 malignant) cases and was non-concordant in 2 cases. Maximum patients with breast lumps presented in the age group of 30-49 years. Carcinoma was seen in 16 (13.3%) cases in age group of 30-80 years. Out of clinical breast examination, mammography and/or ultrasonography, and fine needle aspiration cytology, diagnostic accuracy of the latter was highest (100%). The Triple Test Score had a diagnostic accuracy of 98.33% and sensitivity, specificity, positive predictive value and negative predictive value of 100% each. The Triple Test was correlated with biopsy reports and yielded comparable diagnostic accuracy. Triple Test assessment is a very useful diagnostic tool for evaluating breast lumps with a diagnostic accuracy of 98.33% as revealed by our study which was further substantiated by histopathology.

Keywords: Palpable Breast Lumps, Fine needle Aspiration Cytology, Triple Test Score.

INTRODUCTION

Breast cancer is the leading cause of morbidity and mortality in women, both in the developed and developing countries. The incidence of breast cancer is increasing in the developing world due to increased life expectancy, adoption of western lifestyles, urbanization and diagnosis in very late stages. Therefore, early evaluation of breast lumps remains the cornerstone of breast cancer control in order to improve outcome and survival [1]. Breast symptoms and signs are common problems in clinical practice. Open biopsy has been the Gold Standard for evaluating suspicious breast lesions but it is painful, expensive and unnecessary in younger age group which have comparatively lower rates of malignancy. Hence, the dogmatic statement 'every breast lump should be excised' should be replaced by a recommendation 'every breast lump should be assessed and clarified'. According to National Institute for Health and Clinical Excellence (NICE) and the

Association of Breast Surgeons guidelines, for patients with symptoms that could be caused by breast cancer, diagnosis is made by Triple Assessment comprising of clinical breast examination (CBE), mammography and/or ultrasonography (USG), and core biopsy and/or fine needle aspiration cytology (FNAC) [2,3]. Hence, the effective diagnosis and management of breast lesions involves multidisciplinary approach to their assessment. The present study was undertaken to evaluate the role of Triple Test in preoperative diagnosis of palpable breast lesions and to evaluate performance characteristics of its components individually and combined keeping histopathological examination as reference standard.

MATERIALS AND METHODS

In this multi institutional prospective study a total of 120 cases, irrespective of age, presenting with palpable breast lesions were studied over a period of

five years, since 2009 to 2014. These patients were subjected to CBE, mammography and/or USG and FNAC in that order. The patients were referred from in-patient and out-patient departments of the hospitals.

Inclusion Criteria:

Patients who presented with palpable breast lesions.

Exclusion Criteria:

1. Patients who presented with breast complaints other than palpable breast lesions and patients in whom no definable mass was detected on physical examination.
2. Patients with mastitis, breast abscesses, ulcerated and fungating breast growths and breast fistulae were excluded as mammography was not possible in such cases.
3. Pregnant females

Informed consent was obtained from the patients before clinical examination and investigations. Thereafter, detailed history of the patient was taken followed by physical examination. The breasts were inspected for asymmetry, skin retraction, ulceration or edema and then palpated. A note was made of the quadrant in which the mass was present. Both the breasts were examined to rule out presence of any additional masses. The axilla was examined for lymphadenopathy. Patients below 40years of age were subjected to USG. Female patients more than 40years were subjected to mammography as well as USG to minimize radiation hazard to reproductive group.

Mammogram: Two views medio-lateral oblique and standard cranio-caudal were obtained using a dedicated mammographic unit.

FNAC: The aspirations were done in the cytology department on out-patient basis. Material was obtained with a fine 22 and 24G needle fitted to 10ml plastic syringe with cameo syringe pistol, which facilitated single handed aspiration. No local anesthesia was required and alcohol scrub was used as an aseptic

precaution. Both dry and wet smears were prepared. The smears thus prepared were stained with Leishman Giemsa stain (dry) while the wet fixed smears were stained by Papanicolaou and Haematoxylin Eosin stains using standard procedures[4].

The three components of Triple Test i.e., CBE, mammography/USG and FNAC findings were categorized as benign, suspicious and malignant. The Triple Test was considered concordant if all the elements indicated a malignant condition or all indicated a benign condition, otherwise it was considered non-concordant. Each parameter was assigned a score of 1, 2 or 3 for benign, suspicious or malignant results, respectively. Triple Test Score(TTS) was calculated as the sum of these scores with a minimum score of 3(concordant benign) and a maximum score of 9 (concordant malignant).A score of ≤ 4 was accepted as benign and ≥ 6 was accepted as malignant. A score of 5 was considered non concordant.

RESULTS

A total of 120 cases of breast lumps were studied. All but one, were women. The age range of the patients varied from 18-80years.Each case was given a TTS wherein each component of Triple Test was given a score of 1, 2 & 3 for benign, suspicious and malignant results, respectively. Out of one hundred twenty, 96 cases had a score of 3 and 14 cases had a score of 9. Two cases scored 8, falling in category of being suspicious as they could not be categorized as clearly benign or malignant, radiologically. Six cases scored 4 and the remaining two scored 5. Assessment of patients by Triple Test yielded 102(85%) benign and 16(13.33%) malignant lumps which were further confirmed through histopathological examination. The Triple Test was non-concordant in 2 (1.66%) patients as there was suspicion of malignancy on CBE, mammography and USG. However, after FNAC and histopathological analysis they were found to be benign (fibrocystic disease).Maximum patients with breast lumps presented in the age group of 30- 39yrs (34.17%) followed by 40-49yrs (21.82%) (Table 1).

Table 1: Age wise distribution of palpable breast lesions

Age in years	No of cases	Percentage of cases (%)
10-19	2	1.67
20-29	23	19.17
30-39	41	34.17
40-49	33	27.50
50-59	11	9.17
60-69	5	4.16
70-79	4	3.33
80	1	0.83

The age group of 30-39 years showed maximum number of benign cases followed by 40-49 years. All the palpable lesions of the breast occurring in the age group of 10-29 years (20.83%) were benign in

this study. Malignancy was seen in 16 (13.33%) in the age group of 35-80 years. The average age of presentation of benign lumps was 35.4 years and that of malignant lumps was 56.7 years (Figure1).

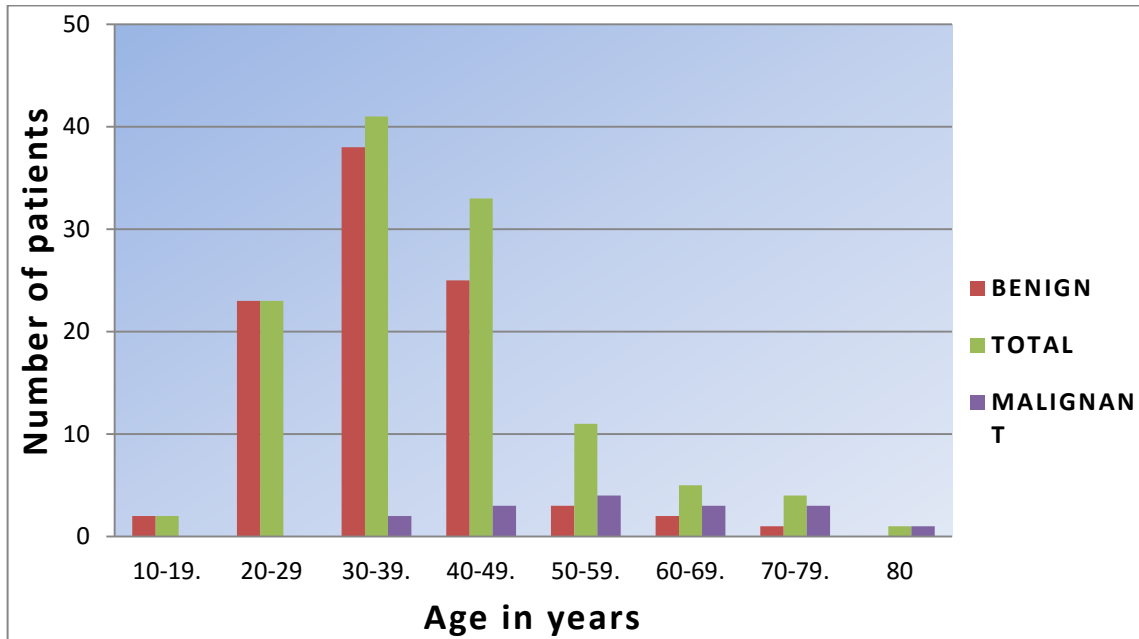


Fig-1: Age wise distribution of benign and malignant palpable breast lesions

It was observed that incidence of malignant lumps increased with advancing age (30-39 years-4%; 40-49 years- 9%; 50-59 years-9.1% ; 60-69 years-60%; 70-80 years- 80%) The statistical significance between presentation of lumps and age was calculated by Fisher Exact Test and was found to be 0. The level of statistical significance was taken as $p \leq 0.05$ [5].

In this study, majority patients presented with upper outer quadrant lumps followed by lower outer quadrant and least in the center (Figure 2). Maximum number of patients presented with lumps and malignancies in upper outer quadrant (Figure 3).

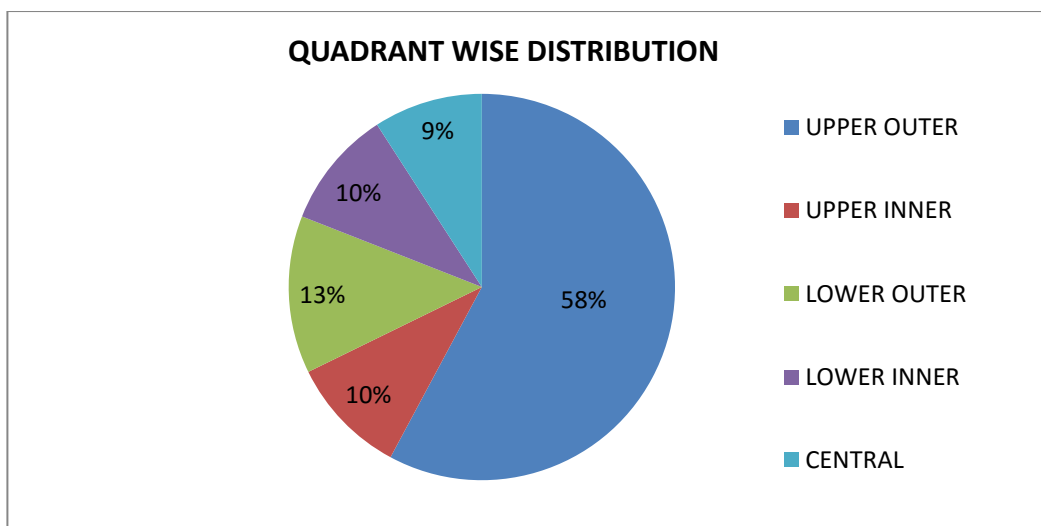


Fig-2: Quadrant wise distribution of palpable breast lesions

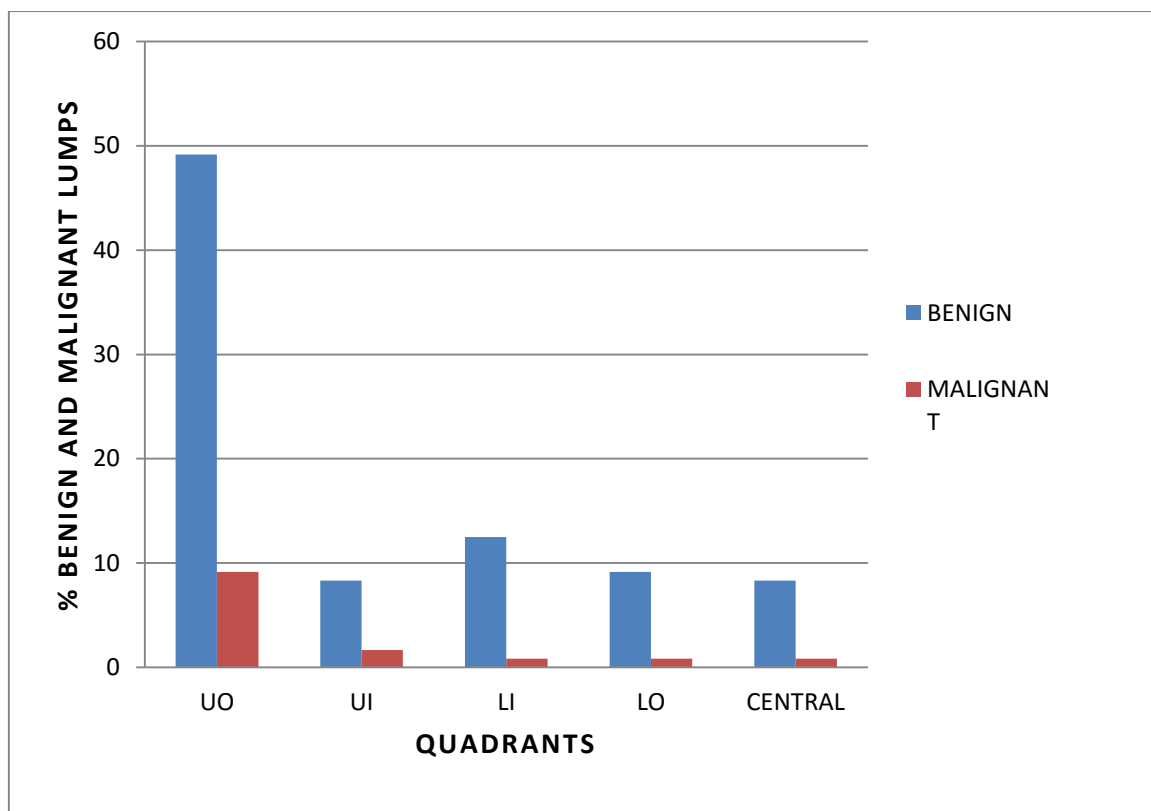


Fig-3: Quadrant wise distribution of benign & malignant palpable breast lesions

Out of the 120 cases, there were 65 cases of fibroadenoma (54.18%), 25 cases of fibrocystic disease (20.84%), 15 cases of invasive carcinoma, no special type (NST; 12.5%), one case of mucinous carcinoma (0.83%), 9 cases of abscess (7.5%), 3 cases of galactocele (2.5%) and one case of benign phyllodes and duct ectasia each (1.67%) on FNAC. One case of invasive carcinoma NST with TTS of 9 (concordant

malignant) was diagnosed in a male patient aged 61years.

It was observed that the most common benign lump was fibroadenoma and among the malignant lumps, invasive carcinoma NST was the commonest. The FNAC results were in 100% agreement with histopathology which was taken as the reference standard (Table 2).

Table 2: Correlation of FNAC, TTS with Histopathology

Diagnosis	FNAC	Triple Test Score (No. of cases)	Histopathology
Fibroadenoma	65	3(64),4(1)	65
Galactocele	3	3(3)	3
Abscess	9	3(8),4(1)	9
Benign Phyllodes	1	3(1)	1
Fibrocystic Disease	25	3(20),4(3),5(2)	25
Duct Ectasia	1	4(1)	1
Mucinous Carcinoma	1	9(1)	1
Invasive Ductal Carcinoma NST	15	9(13),8(2)	15

FNAC= Fine Needle Aspiration Cytology, NST= No Special Type

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and

diagnostic accuracy of CBE, mammography, USG, FNAC and TTS were calculated (Table 3).

Table 3: Diagnostic tool and statistical analysis

Diagnostic Tool	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)
CBE	100	96.16	80	100	96.67
Ultrasonography	100	95.19	76.19	100	95.83
Mammography	100	89.47	78.95	100	92.45
FNAC	100	100	100	100	100
Triple Test	100	100	100	100	98.33

PPV = Positive Predictive Value, NPV = Negative Predictive Value, CBE= Clinical breast examination, FNAC = Fine needle Aspiration Cytology

In the present study, all the diagnostic tools showed 100% sensitivity and negative predictive values. However, FNAC showed the highest diagnostic accuracy followed by Triple Test, both of which were comparable. Further, in addition to USG, mammography was performed in female patients of 40years and above. Both the imaging techniques exhibited good correlation.

DISCUSSION

Annually, over 100,000 cases of breast cancer are diagnosed in India leading to increased health care burden [6]. This rising trend has been observed across all regions of India and in all age groups but more so in the young (< 45 years) [7]. In our study also, 36.7% malignancies presented in the age group 40-49years. In general, breast cancer has been reported occurring a decade earlier in Indian patients compared to their western counterparts. More than 80% of Indian patients are younger than 60 years of age. The average age of breast cancer patients has been reported to be 50–53 years as per National Cancer Registry Program [6]. The majority of breast cancer patients in western countries are postmenopausal and in their 6th and 7th decades. In our study, average age of presentation of malignant lumps was 56.7 years which was a decade earlier than the western counterparts. Since the incidence of breast cancer is rising in the Indian subcontinent, our study was aimed to assess the role of Triple Test in preoperative evaluation of palpable breast lesions and to diagnose malignant lumps.

Our prospective study of Triple Test on 120 cases and its confirmation by histopathology showed Triple Test to be highly sensitive (100%), specific (100%) and having high PPV (100%), NPV (100%) and diagnostic accuracy (98.33%). Since its diagnostic accuracy was higher for malignant lesions, it can be used for definitive treatment especially, in our scenario, where 'frozen section' facilities may not be available routinely [8]. Among the 120 patients, 70 (58%) patients presented with a lump in the upper outer quadrant of breast, followed by 15 (13%) in the lower

outer quadrant and the remaining 37(29%) in other quadrants and central region of breast. Similar observations were reported in other studies where the majority of breast lumps were found in the upper outer quadrant [9-11]. The relative higher occurrence of lumps in the upper outer quadrant of breast could be due to presence of much of the epithelial tissue of breast in this quadrant [12].

Clinical Breast Examination: Digital palpation of the breast is effective in detecting masses and can help determine whether a mass is benign or malignant. In this study, sensitivity, specificity, PPV, NPV and diagnostic accuracy of CBE were 100%, 96.16%, 80%, 100% and 96.67%, respectively.

Kharkwal *et al* in their studies found the above as 75%, 83.3%, 75% and 80%, respectively[13]. Further, in another study the sensitivity, specificity, PPV, NPV and accuracy of the CBE were reported as 66.6%, 100%, 100%, 90%, and 91.6%, respectively [9]. The review of the literature, shows the sensitivity of the CBE ranging from 21% to as high as 100% and the specificity from 50% to 100%.

In the current study, the high sensitivity could be due to inclusion of patients with symptoms of breast lumps, either self-detected or by a physician. Despite its accuracy, CBE alone is not adequate for definitive diagnosis of breast cancer. Further evaluation is required in all patients with breast masses including follow-up examinations, imaging, and tissue sampling[14].

Ultrasonography: Ultrasonography is the well accepted initial imaging technique in young women for characterizing breast lumps because of dense breast tissue. In our study, USG favored malignancy in 14 patients which was confirmed by FNAC and histopathology. Out of 7 suspicious cases, 5 turned out to be benign and two were malignant on FNAC as well as histopathology. Thus, for USG, sensitivity, specificity, PPV, NPV and diagnostic accuracy were

100%, 95.19%, 76.19%, 100% and 95.83%, respectively. These findings compared well with studies by Pande *et al.* who reported the sensitivity, specificity, PPV and NPV of USG as 95%, 94.1%, 95.5%, and 93.7%, respectively [15]. A similar USG study by Jan *et al.*, showed the above parameters as 100%, 96.4%, 66.7% and 100%, respectively [16].

The present study showed a diagnostic accuracy of 95.83% and the remaining 4.17% lesions which were reported suspicious turned out to be benign on FNAC and histopathology. Thus, a suspicious result on USG does not completely establish a possibility of malignancy. In case of a suspicious report, additional testing modalities should be used to rule out malignancy. A specificity of 100% and a positive predictive value of 100% means that a USG report can be relied upon for treatment decisions when a malignant result is reported.

Mammography: Mammography aids in detecting breast mass with malignant features earlier along with screening for synchronous lesions in the ipsilateral and contra-lateral breast. We performed mammography along with USG on 53 women with palpable breast lumps who were ≥ 40 years old as few studies showed that USG could be combined with mammography to increase sensitivity and specificity[17,18]. In this study, the sensitivity, specificity, PPV, NPV and

diagnostic accuracy of mammography were 100%, 89.47%, 78.95%, 100% and 92.45% respectively. On review of the literature, the above data for mammography ranged between 69-100%, 70-90%, 53-86%, 80-100% and 80-90% respectively [14,19-21]. The sensitivity, specificity, PPV, NPV and diagnostic accuracy of USG in conjunction with mammography were 100%, 86.84%, 75%, 100% and 90.56%, respectively in the current study and were comparable with those of mammography alone. Hence, performing both imaging modalities did not substantiate the prediction. More accurate prediction is added by FNAC/biopsy. Breast imaging is still valuable but in settings where health resources are limited, single breast imaging (only either USG or mammography) is adequate for cancer diagnosis [22]. It is, therefore, unnecessary to perform both imaging techniques. Accuracy of the diagnosis may be improved by FNAC.

Fine Needle Aspiration Cytology: The sensitivity, specificity, PPV, NPV and diagnostic accuracy of FNAC of breast lumps in our study were 100% implying that this technique is the most reliable diagnostic tool out of the three parameters of the Triple Test. It favored malignancy in 16 cases and benign etiology in 104 cases which were in agreement with histopathology. Our findings were comparable to earlier reports (Table 4).

Table 4. Statistical analysis of FNAC as a diagnostic test in various studies

Study	Sensitivity (%)	Specificity(%)	PPV(%)	NPV (%)	Diagnostic Accuracy
Present Study	100	100	100	100	100
Rajan <i>et al</i> [23]	96.60	100	100	-	-
Karkwal <i>et al</i> [14]	94.70	98.30	97.30	96.60	96
Mohammed <i>et al</i> [24]	90.62	100	100	95.08	-

PPV = Positive Predictive Value, NPV = Negative Predictive Value

Triple Test: Our investigations showed, the sensitivity, specificity, PPV and NPV of Triple Test as 100% each while it yielded a diagnostic accuracy of 98.33%, which

compared very well with findings of other studies (Table 5).

Table-5: Statistical analysis of TTS in various studies

Study	Sensitivity (%)	Specificity(%)	PPV(%)	NPV (%)	Diagnostic Accuracy
Present Study	100	100	100	100	100
Karkwal <i>et al</i> [14]	100	100	100	100	-
Smita <i>etal</i> [19]	97.44	100	100	96	-
Mande <i>et al</i> [25]	100	100	100	-	-

PPV = Positive Predictive Value, NPV = Negative Predictive Value

Out of the components of Triple Test, FNAC had the highest diagnostic accuracy as compared to USG/Mammography (Figure 4).

In our study, lumps which scored ≤ 4 on Triple Test were concordant benign and the ones scoring ≥ 8

were concordant malignant while two earned a score of 5 each, which turned out to be benign (Fibrocystic Disease). The above findings were confirmed by histopathology. Though, in the present work none of the lumps scored 6 or 7, these may be placed as highly

suspicious for malignancy and be confirmed by histopathology.

CONCLUSION

Patients with palpable breast lumps should be evaluated by Triple Test. When all the three components of Triple Test are concordant i.e either concordant benign or concordant malignant, it yields 100% diagnostic accuracy. Amongst the three components of Triple Test, FNAC is most reliable. A Triple Test Score of 5 has low predictive value and such cases would require open biopsy for confirmation of the diagnosis.

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