Original Research Article

Diagnostic challenges during recognition of isolated posterior wall myocardial infarction

Pankaj Gupta[1], Ruchika Solanki[2], Mimansha Patel[3*],Sanjay Deb[4], Radhika Vashistha[5],Atul Sharma[6]

[1]Chief Surgical Pathologist,Blupath Lab,Ex DNB resident Dharamshila Cancer Hospital, New Delhi,India
[2]Private practice,India
[3]Reader, Department of Oral Pathology and Microbiology, Triveni Dental College, Bodri, Bilaspur, Chhattisgarh,India
[4]Sr Consultant Pathology & Oncopathology,India
[5] Pathologist,Panipat,Haryana,India
[6]Radiologist,Private practice,India

Received: 12-04-2021 / Revised: 28-05-2021 / Accepted: 18-06-2021

Abstract

Background: It is observed that seven percent of all the cases of breast cancer of whole world belong to our country. If our country is being considered then it has been found that more than twenty percent of all cancers affecting females is the breast cancer. The main concern regarding the management of breast carcinoma is the non homogenous characteristics of the breast tumour. Among the various classifications to reduce this non homogenecity in breast carcinoma the most accepted classifications has been based on the genetic characteristics of the tumours. In recent times there has been several studies has been conducted in other populations focussing on the immune histochemistry markers like Ki 67, cytokeratin 5/6, human epidermal growth factor receptor (HER) and progesterone receptor (PR) in breast carcinoma for assessing molecular and histological subtypes of breast cancer.**Aim:** To evaluate molecular subtypes and histological subtypes of breast cancer based on immune histochemistry markers for assessing the behaviour and disease aggressiveness **Methods and Materials:** Immunohistochemistry was performed using four main markers ER, PR, HER2, and Ki67 to classify them into four molecular subtypes Luminal A, Luminal B, TNBC and HER2. An additional marker CK5/6 was used to further classify TNBC into Basal like and Non Basal like. The characteristics of two subtypes Basal like and non basal like TNBC were analyzed separately. These molecular subtypes and tumour histological subtypes were correlated with clinocopathological parameters viz. Age, menopausal status, laterality (right or left), tumour size, tumour grade, LVI, necrosis, stromal reaction, lymph node status, pathological T stage(pT), pathological N (pN) stage, Nottingham's prognostic index (NPI).**Results:** In present study the age range of patients was between 28 to 80 years, with majority of patients in age group of 50 to 59 years. The mean age of presentation of histological subtypes was Infiltrating duct carcinoma, no special type (IDC-NST) – 53.56 years Infiltrating lobular carcinoma (ILC) – 54.08 years. Other histological subtypes – 57.07 years. In our study out of 278 patients, 105 were in premenopausal group and 172 were in postmenopausal group. Molecular subtypes was found to be more associated with aggressiveness of disease as compared to histological types. **Conclusion:**In comparison to histological subtypes, molecular subtypes can be a better tool for analysing the behaviour and disease aggressiveness of breast cancer, according to the findings of this study. We recommend that molecular classification be performed on all breast cancers and that it be used in conjunction with histological classification. It would be premature to dismiss histological classification at this time.

Keywords: Breast carcinoma, Molecular subtypes, histological subtypes

Introduction

According to recent reports breast cancer is considered as among the most frequent tumours affecting the females. However some cases of carcinoma of breast has been observed in males also. In our country the frequency of the breast carcinoma is also increasing day by day. If the recent reports are to be believed then cancer of breast is considered as the most common cancer in the females residing in the metro cities while if the rural areas are considered then it was found that the breast cancer was second most common cancer affecting females. In rural areas the carcinoma of the cervix is the most common cancer affecting females which is followed by the cancer of breast [1,2].It is observed that seven percent of all the cases of breast cancer of whole world belong to our country. If our country is being considered then it has been found that more than twenty percent of all cancers affecting females is the breast cancer. If the epidemiological data are being considered then cervical cancer was most common among females in our country one decade before. But nowadays breast cancer has overtaken the cervical cancer as the most common cancer affecting females in many parts of our country. The reason for such recent condition was change in the lifestyles of the human population and greater impact of western culture in larger cities of our country[3,4].In order to develop good treatment options and descrease the number of deaths due to breast cancer there is need to have better knowledge about the etiopathogenesis of the breast carcinoma. Nowadays the recent concept believes that breast carcinoma is not a single disease but it is non homogenous collection

**Correspondence*
Dr. Mimansha Patel
Reader, Department of Oral Pathology and Microbiology, Triveni Dental College, Bodri, Bilaspur, Chhattisgarh,India
E-mail: mimanshapatel_24@yahoo.com

of pathological entities having different morphological and clinical features. Tumours of breast exhibiting different clinical features may have same histopathological features. Besides there may be a condition in which breast tumours having similar histopathological features may have different response to treatment and different aggressiveness. The reason for such variations is due to drawbacks in the recent classification of tumours of breast which is mainly based on the morphological features[5,6].It has been reported that separate strategy for separate features of the breast carcinoma can helpful in improving the outcome of the management of the breast cancer. Currently there are some drug regimens which are being used in routine management of breast carcinoma. These are Trastuzumab, Aromatase inhibitors and Tamoxifen. It is believed that if the knowledge about the molecular characteristics of breast carcinoma will increase then there can be introduction of new drug regimens which will further increase the outcome of the management of this cancer. Some of the drug regimens for breast cancer are undergoing clinical trials nowadays. These drugs are being developed on the basis of knowledge obtained after studying the molecular and histological characteristics of the breast carcinoma. Some of these new drug regimens are inhibitors of HER 1- RAS pathway and inhibitors of poly ADP ribose polymerase enzyme[7,8].The main concern regarding the management of breast carcinoma is the non homogenous characteristics of the breast tumour. This heterogenecity of breast carcinoma has been a matter of debate since several years. Several classifications has been proposed in past which have helped in developing a proper classification system which have improved the treatment options and prognosis of the breast carcinoma. Among these classifications the most accepted classifications has been based on the genetic characteristics of the tumours[9,10].In recent times there has been several studies has been conducted in other populations focussing on the immune histochemistry markers like Ki 67, cytokeratin 5/6, human epidermal growth factor receptor (HER) and progesterone receptor (PR) in breast carcinoma. In our country very less number of studies has been conducted focussing on the molecular subtypes of breast carcinoma[11,12].Therefore this study was carried out with the aim of determination of molecular profiles of breast carcinomas using expression of ER, PR, HER2, CK5/6 and Ki67.

Materials and methods

After receiving approval from the Scientific and Ethics Committee, the study was carried out in the Department of Pathology at a tertiary level hospital and research centre in north India. From 2009 to 2020, a total of 278 instances of invasive breast carcinomas were included in the study. Tissue blocks that had been formalin fixed and paraffin embedded (FFPE) were obtained. Molecular subtypes were identified using IHC surrogate markers, as described by the 12th St. Gallen consensus. Using the CK5/6 antibody, the triple negative subtype was further divided into two categories: basal and non-basal.

Study Site :Study was conducted at Department of Pathology of tertiary level hospital and research centre.

Study Population:Study was conducted at tertiary centre of North India, which covers population from north and north east.

Study Design :A combined retrospective and prospective study from year 2009 to 2020, with multivariable analysis of 278 breast cancer specimens.

Study Duration : The study was conducted on FFPE blocks of carcinoma breast specimen, available in the Department of Pathology, diagnosed between Jan 2009 to July 2020.

Sample Size :A total of 278 instances of breast cancer tissues were studied, all of which were diagnosed at our hospital. According to the statistical formula n=4(pq)/E2, where p is the percentage prevalence of breast cancer in the study population, q = 1-p, and E is the total permissible error, the sample size was appropriate for a total acceptable error of 5% and a confidence level of 95%. A random sample was taken.

Inclusion Criteria

Our investigation contained FFPE blocks of the following specimens, which were evaluated and analysed.

1.Invasive carcinoma found in breast conservation surgery specimens

2. Invasive cancer specimens from modified radical mastectomy

3.Others with invasive carcinoma (radical mastectomy, toilet mastectomy)

Exclusion Criteria

The following scenarios were ruled out:

1. Only in situ breast cancer specimens
2. Specimens of male breast cancer
3. Biopsy specimens that have been truly cut

Methodology

The FFPE blocks have been located. The following are the results of H& E and IHC staining and interpretations:

Interpretation of hematoxylin and eosin-stained slides

The following parameters were assessed microscopically on these H&E stained sections.

Tumor subgroups based on histology (IDC, ILC or others)
Tumour histological grade (Modified Blood Richardson Grade)
Presence or absence of necrosis
Presence or absence of stromal reaction
Presence or absence of lymph vascular invasion

Interpretation of IHC results

Hormone receptors (Estrogen and Progesterone): For reporting oestrogen and progesterone receptor expression, we followed the American Society of Clinical Oncologists (ASCO) and College of American Pathologists (CAP) recommendations. The recommendations state that

• Carcinomas that contain at least 1% positive cells should be considered receptor positive.

• Carcinomas with less than 1% positive cells should be ruled out as receptor-negative.

The fraction of positive cells and the degree of immunoreactivity are used to quantify ER and PR.

• The number of positive cells might be expressed as a percentage or as discrete groups.

• The degree of nuclear positivity is referred to as intensity (i.e. pale to dark). The amount of protein can influence the intensity.

HER2 interpretation

This was carried out according to guidelines given in the table below.

Table 1:HER2 interpretation

Results	Criterion
Negative (Score 0)	No staining observed Or Incomplete, faint/barely perceptible membrane staining in less than 10% invasive tumour cells
Negative (Score 1+)	Incomplete, faint/barely perceptible membrane staining in greater than10% invasive tumour cells
Equivocal	Incomplete and/or weak to moderate circumferential staining in greater than

(Score 2+)*	10% of invasive tumour cells Or Complete, intense, circumferential membrane staining in less than or equal to 10% of invasive tumour cells
Positive (Score 3+)	Complete, intense, circumferential membrane staining in greater than 10% of invasive tumour cells

Ki67 Interpretation

- Assessment of Ki67 was done using guidelines laid down by International Ki67 in Breast cancer working group. According to these guidelines
- Only nuclear staining is to be considered as positive.
- Intensity of staining is not relevant.
- At least three high power fields (40x) should be selected to represent spectrum of staining seen on initial overview of whole section
- Scoring should involve counting of minimum of 500 malignant invasive cells
- If there are clear hot spots, data from these should be included in final results (hot spots are defined as the areas in which Ki67 staining is particularly prevalent, may occur in otherwise homogenously stained sample).
- Ki67 is reported as percentage positivity with a maximum score of 100% and lowest of zero percent.
- We used an internal control of nonmalignant cells and mitotic figures as a quality indicator.

The precautions taken while handling and processing of specimen to avoid false results are same as that for androgen receptors.

Interpretation of CK5/6

- All tumour cells showing cytoplasmic staining are taken as positive for CK5/6.
- Control used is a known case of Squamous cell carcinoma
- The precautions taken while handling and processing of specimen to avoid false results are same as that for androgen receptors.

The information was gathered using the study's proforma. Immunohistochemistry was used to categorise them into four molecular subtypes: Luminal A, Luminal B, TNBC, and HER2 utilising four primary markers: ER, PR, HER2, and Ki67. TNBC was further classified as Basal like and Non Basal like using the additional marker CK5/6. The characteristics of two subtypes of TNBC were studied separately: basal like and non basal like TNBC. To identify these subtypes, we used the St. Gallen consensus classification system, which divides Luminal B into two subgroups: Luminal B Her2 negative and Luminal B Her2 amplified. When performing statistical analysis, both of these groupings were treated as different entities. Age, menopausal status, laterality (right or left), tumour size, tumour grade, LVI, necrosis, stromal reactivity, lymph node status, pathological T stage(pT), pathological N (pN) stage, Nottingham's prognostic score were all connected with these molecular and histological subtypes (NPI).(Figure 1 to 6)

Statistical methods

Number of patients and percentage of patients were used to compare categorical variables between groups using Pearson's Chi Square test for independence of attributes and Fisher's exact test. The mean and standard deviation of continuous variables were calculated and compared across groups using the one-way ANOVA test. The analysis was carried out using the statistical software SPSS version 20. A 5% alpha level was used, which meant that any p value less than 0.05 was considered significant.

Results

Clinico-pathological characteristics

Age :Patients in this study ranged in age from 28 to 80 years old, with the bulk of patients in the 50 to 59 year old age group. The average age of histological subtype presentation was 53.56 years for infiltrating duct carcinoma, no special type (IDC-NST). 54.08 years with infiltrating lobular cancer (ILC). 57.07 years for other histological subtypes. (Table 1) This distribution was not significant statistically. (p=0.558, F=0.585, AVOVA.) The mean age of presentation of molecular subtypes was as follows: Luminal A – 60.06 years. Luminal B Her2 negative – 55.26 years. Luminal B Her2 amplified – 51.41 years. Triple negative Breast cancer (TNBC) – 49.76 years HER2 type – 52.17 years. This distribution showed a significant statistical difference when analyzed by analysis of variance (ANOVA) with p value equal to 0.02. It was found that the mean age at presentation of TNBC is significantly lower as compared to other subtypes whereas Luminal A subtypes is more commonly seen in the older age group.

Menopausal status : To evaluate the relationship between molecular subtypes and menopausal status, researchers divided the participants into two groups: premenopausal and postmenopausal. Out of the 278 individuals in our study, 105 were premenopausal and 172 were postmenopausal. This was the pattern of molecular subtype distribution. In Luminal A, 80.9 percent (55 of 68) of the participants were postmenopausal, while 19.1 percent (13 of 68) were premenopausal.

Among Luminal B HER2 negative, 68.4 percent (26 of 38) were in postmenopausal group and 31.6 percent (12 of 38) were in premenopausal group. Out of 59 Luminal B HER2 amplified, 52.5 percent (31) were postmenopausal and 47.5 percent (28) were premenopausal. Among 66 patients of TNBC subtype, 48.5 percent (32)were postmenopausal and 51.5 percent (34) were premenopausal. In HER2 subtype 61.7 percent (29 of 47) were postmenopausal and 38.3% (18 of 47) were premenopausal. (Table 3)

Laterality : In our study 46.4 percent (129) patients had right sided breast cancer and 53.6 percent (149) patients had left sided breast cancer. There was no significant statistical difference in the distribution of histological subtypes and molecular subtypes with these subgroups (p =0.186 for histological subtypes and p = 0.177 for molecular subtypes by Pearson Chi square).

Tumour size: Most of the patients in our study had tumour size between 2-5 cm 72.7 percent (202), 11.2 percent (31) had tumour size less than 2 cm and 10.8 percent (30) patients had size more than 5 cm. In 5.4 percent (15) tumour size could not be assessed due to presence of diffuse tumour as a result of neo adjuvant chemotherapy. (Table 4)

Histological Subtype : Infiltrating duct carcinoma (No special type) (IDC-NST) was the most common histological subtype constituting 85.6 percent (238) out of 278 cases, followed by infiltrating lobular carcinoma (ILC) 9.4 percent (26) and others 5.0 percent (14) including 4 mucinous carcinomas, 2 each of papillary, medullary, secretory carcinomas and 1 each of infiltrating tubular, metaplastic, mucin secreting and poorly differentiated carcinomas. The data was compared with molecular subtypes using Pearson Chi-square test, and the result were not significant statistically (p=0.50).

Ki67 Proliferation Index : In present study out of 31.3 percent (87 of 278) tumours had Ki67 less than 14 percent and 68.7 percent (191 of 278) tumours had Ki67 more than 14 percentKi67 is incorporated as an important criterion to differentiate Luminal A from Luminal B Her2 negative tumours. In tumours which are positive for ER and /or PR and are negative for HER2, the Ki67 index is evaluated. Those with Ki67 >14 percent are classified as Luminal B HER2 negative and those with Ki67 <14 percent are classified as Luminal A. We analyzed both the histological subtypes and the molecular subtypes with two categories of Ki67 (<14% and >14%).By Pearson Chi

square test there was no significant statistical association between histological subtypes and Ki67 index (p=0.08)

All Luminal A tumours (100%) had Ki67 less than 14 percent and all Luminal B Her2 negative tumours (100%) had Ki67 greater than 14 percent. Among Luminal B Her2 amplified 81.4 percent (48) had Ki67 greater than 14 percent and 18.6 percent (11) had Ki67 less than 14 percent. Most of the TNBC patients, 93.9 percent (62 of 66) had Ki67 greater than 14 percent and rest had Ki67 less than 14 percent (6.1%, 4 of 66). Among HER2 subtype 91.5 percent (43 of 47) tumours had Ki67 more than 14 percent and 8.5 percent (4 of 47) had Ki67 less than 14 percent. This distribution had a significant p value of 0.004 by Pearson Chi square test.

Follow up analysis : Follow up data could be obtained for 170 cases out of 278 in our study. Among these 44 were in Luminal A subtype, 21 were in Luminal B HER2 negative subtype, 40 were in Luminal B HER2 amplified subtype,31 were in TNBC subtype, 34 were in HER2 subtype. Among Luminal A subtypes, one patient developed mediastinal and right axillary node metastasis in a period of 6 months from diagnosis. Among Luminal B HER2 negative there was no local or systemic recurrence.

Among Luminal B HER2 amplified, two patients developed leptomeningeal metastasis one at 8 months other at 36 months. Two patients developed skeletal metastasis one at 12 months other at 24 months. Among TNBC one patient developed liver metastasis at 26 months and one developed leptomeningeal metastasis at 6 months. Among HER2 subtype, one patients had wide spread metastatic disease at presentation. One patient developed liver metastasis at 12 months. One patient developed both lung and liver metastasis at 12 months. One patient developed leptomeningeal metastasis at 12 months. One patient developed lung metastasis at 36 months

Table 2: Age And Histological Subtypes

	IDC	ILC	Others	ANOVA
Mean	53.56	54.08	57.07	0.558
Median	53.00	55.00	61.50	
STD. Deviation	11.46	13.16	16.16	

Table 3: Age and Molecular Subtypes

	Luminal A	Luminal B		TNBC	HER2	ANOVA
		HER2 (-)	HER2 (+)			
Mean	60.06	55.26	51.41	49.76	52.17	0.02
Median	61.00	55.50	50.00	49.00	50.00	
STD. Deviation	11.819	12.229	11.385	10.726	10.248	

Table 4: Menopausal Status with Molecular Subtypes

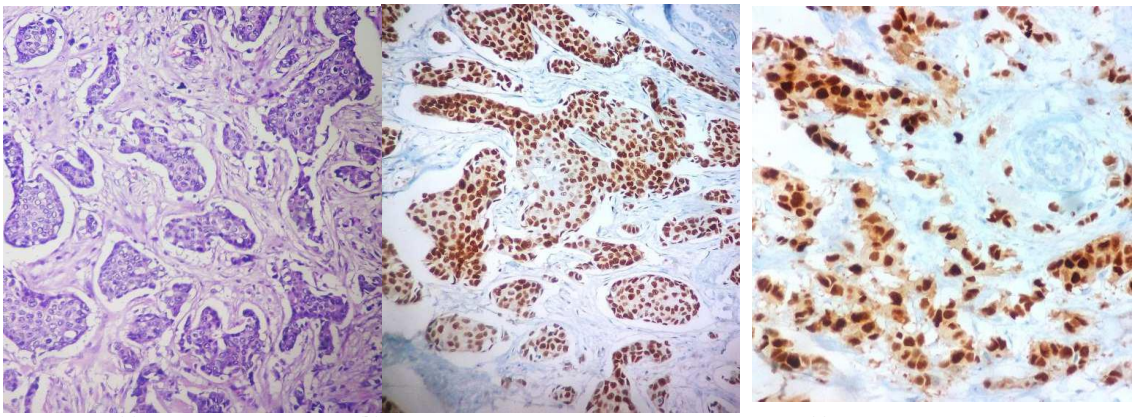
Menopausal status	Molecular Subtypes											Chi Square Test
	Luminal A (n=68)		Luminal B				TNBC(n=66)		HER2(n=47)		Total	
			HER2 (-) (n=38)		HER2 (+) (n=59							
	No.	Percent	No.	Percent	No.	Percent	No	Percent	No	Percent		
Premenopausal (≤ 50 years)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] 0.001
Postmenopausal (>50 years)	55	80.9%	26	68.4	31	52.5	32	48.5	29	61.7	173	

Table 5: Molecular Subtypes With Tumour Size

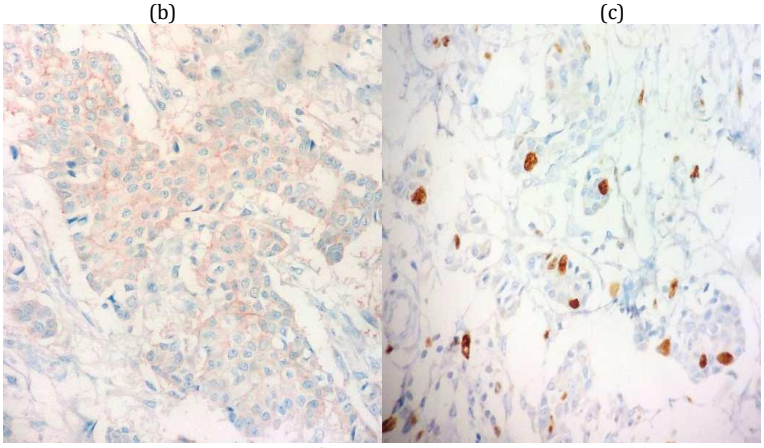
Tumour size (cm)	Molecular Subtypes											Chi Square Test
	Luminal A (n=68)		Luminal B				TNBC (n=66)		HER2 (n=47)		Total	
			HER2 (-) (n=38)		HER2 (+) (n=59							
	No.	Percent	No.	Percent	No.	Percent	No	Percent	No	Percent		
<2	10	14.7	3	7.9	6	10.2	5	7.6	7	14.9	31	0.04
2-5	52	76.5	31	81.6	41	69.5	48	72.7	30	63.8	202	
>5	5	7.4	3	7.9	7	11.9	10	15.2	5	10.6	30	
Undetermined	1	1.5	1	2.6	5	8.5	3	4.5	5	10.6	15	

Table 6: Distribution of Molecular Subtypes With Ki67 Index

Ki67	Molecular Subtypes											Chi Square Test
	Luminal A(n=66)		Luminal B				TNBC(n=66)		HER2(n=47)		Total	
			HER2 (-) (n=38)		HER2 (+) (n=59							
	No.	Percent	No.	Percent	No.	Percent	No	Percent	No	Percent		
<14%	68	100%	0	0	11	18.6	4	6.1	4	8.5	87	0.004
>14	0	0	38	100	48	81.4	62	93.9	43	91.5	191	

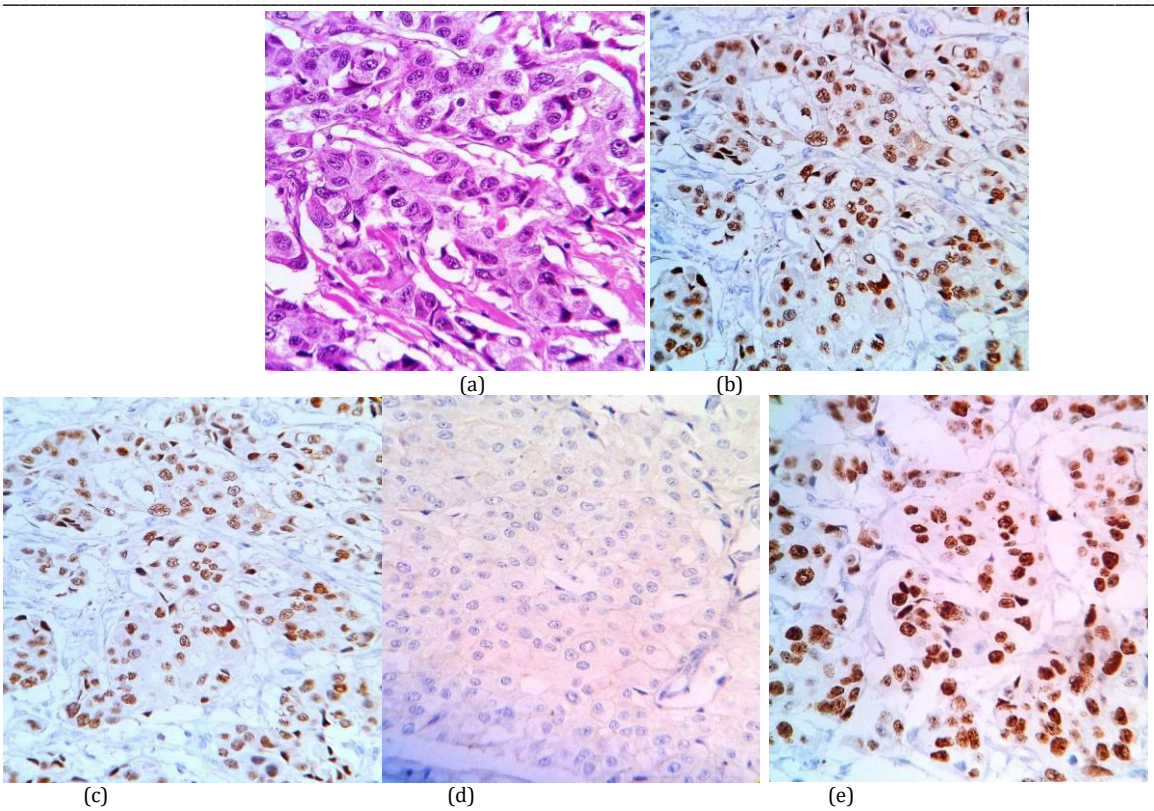


(a) (b) (c)



(d) (e)

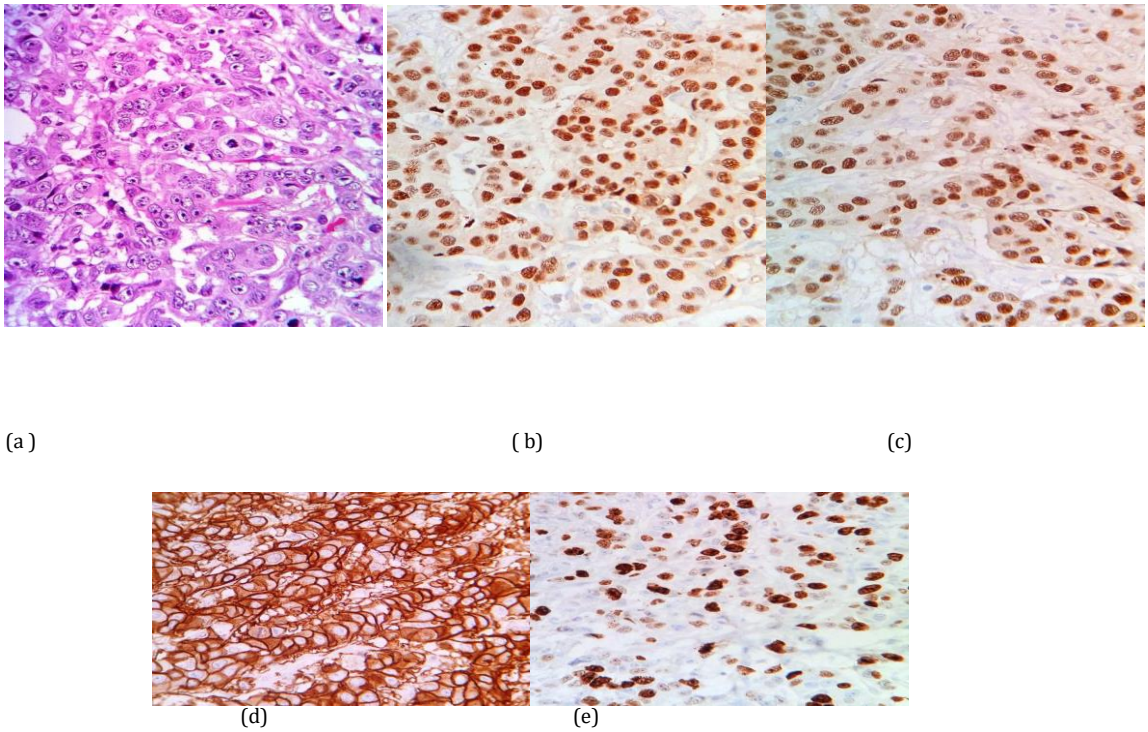
Fig 1: Luminal A. Microphotographs showing Morphology and IHC (a)Hematoxylin and Eosin; (b) IHC stain, ER (c) IHC stain, PR (d) IHC stain,HER2; (e) IHC stain Ki67 (< 14); X 400



(a) (b)

(c) (d) (e)

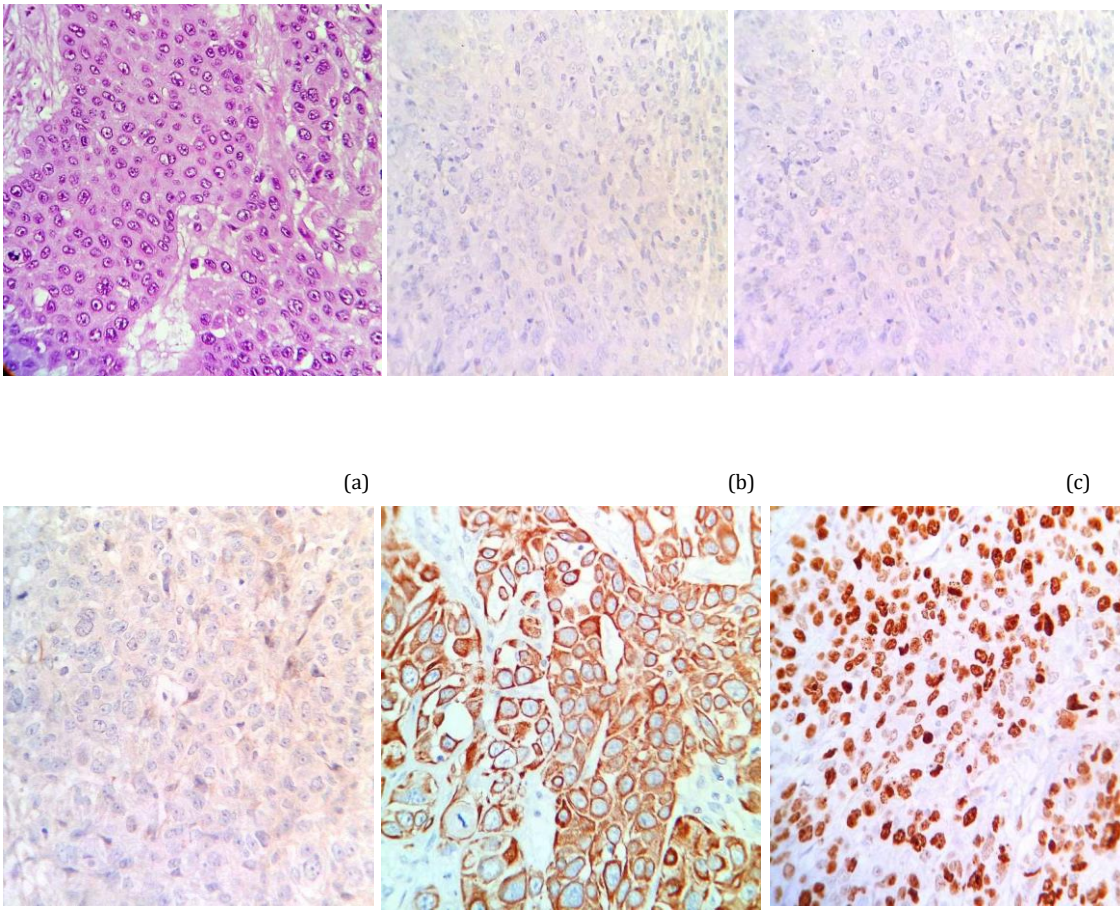
Fig 2: Luminal B HER2 Negative. Microphotographs showing Morphology andIHC (a) Hematoxylin and Eosin; (b) IHC stain, ER (c) IHC stain, PR (d) IHC stain,HER2; (e) IHC stain Ki67; X 400



(a) (b) (c)

(d) (e)

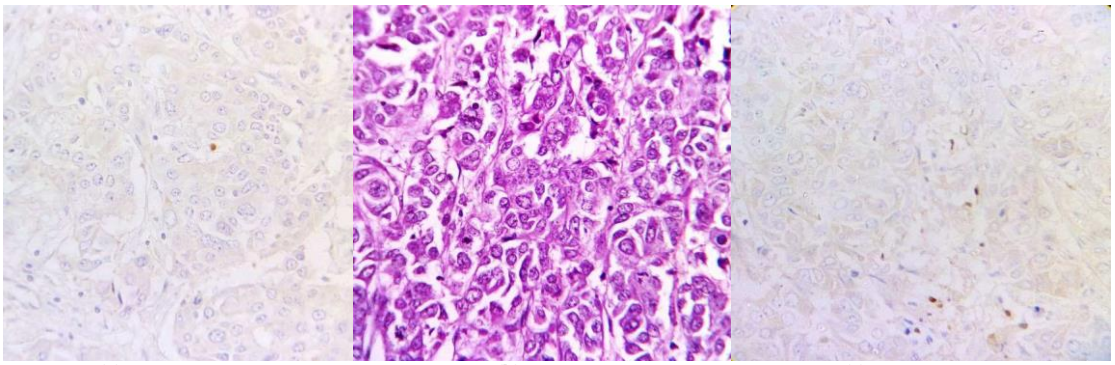
Fig 3: Luminal B HER2 Amplified (HER2+). Microphotographs showing Morphology and IHC (a) Hematoxylin and Eosin; (b) IHC stain, ER (c) IHC stain, PR (d) IHC stain,HER2; (e) IHC stain Ki67; X 400



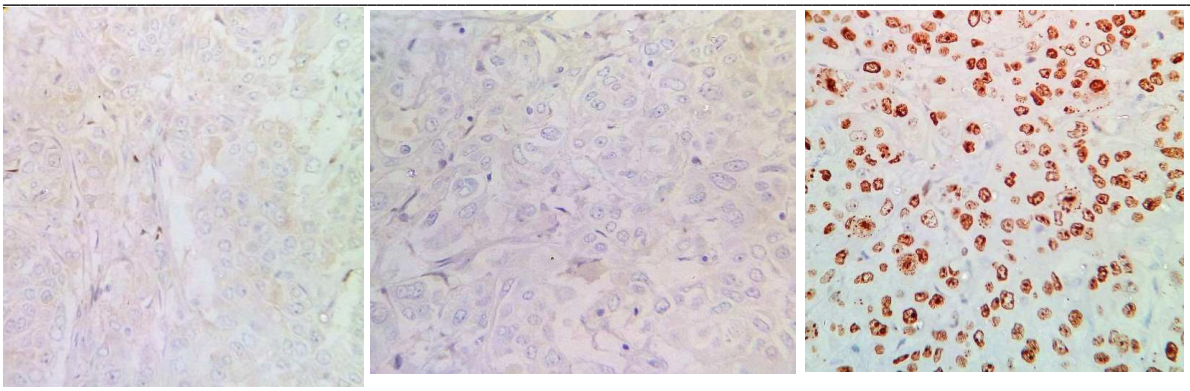
(a) (b) (c)

(d) (e) (f)

Fig 4: Basal like (TNBC). Microphotographs showing Morphology and IHC (a) Hematoxylin and Eosin; (b) IHC stain, ER (c) IHC stain, PR (d) IHC stain,Her2; (e) IHC stain, CK5/6 (f)IHC stain Ki67; X 400

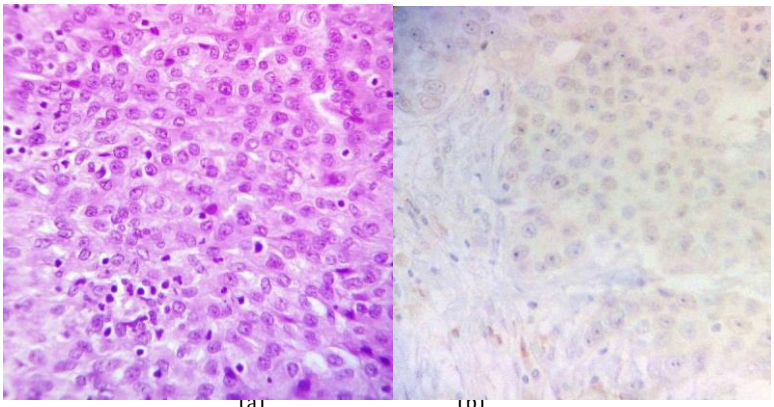


(a) (b) (c)

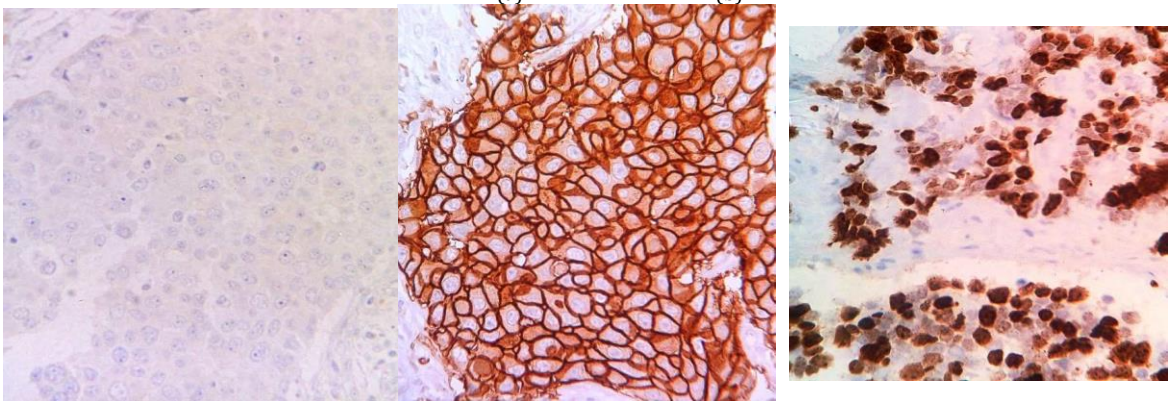


(d) (e) (f)

Fig 5: Non Basal like (TNBC). Microphotographs showing Morphology and IHC (a) Hematoxylin and Eosin; (b) IHC stain, ER (c) IHC stain, PR (d) IHC stain,Her2; (e) IHC stain, CK5/6 (f)IHC stain Ki67; X 400



(a) (b)



(c) (d) (e)

Fig 6: HER2 Types. Microphotographs showing Morphology and IHC (a) Hematoxylin and Eosin; (b) IHC stain, ER (c) IHC stain, , PR (d) IHC stain,Her2; (e)IHC stain Ki67; X 400

Discussion

If the recent reports are to be believed then cancer of breast is considered as the most common cancer in the females residing in the metro cities while if the rural areas are considered then it was found that the breast cancer was second most common cancer affecting females. In rural areas the carcinoma of the cervix is the most common cancer affecting females which is followed by the cancer of breast. It is observed that seven percent of all the cases of breast cancer of whole world belong to our country. When our country is being considered then it has been found that more than twenty percent of all cancers affecting females is the breast cancer[13,14]On considering the epidemiological data it has been found that a cervical cancer was most common among females in our country one decade before. But nowadays breast cancer has overtaken the cervical cancer as the most common cancer affecting females in many parts of our country. The reason for such recent condition was change in the lifestyles of the human population and greater impact of western culture in larger cities of our country.In order to develop good treatment options and descrease the number of deaths due to breast

cancer there is need to have better knowledge about the etiopathogenesis of the breast carcinoma[15,16].This heterogenecity of breast carcinoma has been a matter of debate since several years. Several classifications has been proposed in past which have helped in developing a proper classification system which have improved the treatment options and prognosis of the breast carcinoma. Among these classifications the most accepted classifications has been based on the genetic characteristics of the tumours[17].In recent times there has been several studies has been conducted focussing on the immune histochemistry markers like Ki 67, cytokeratin 5/6, human epidermal growth factor receptor (HER) and progesterone receptor (PR) in breast carcinoma. In our country very less number of studies has been conducted focussing on the molecular subtypes of breast carcinoma. Therefore this study was carried out with the aim of determination of molecular profiles of breast carcinomas using expression of ER, PR, HER2, CK5/6 and Ki67[18].

In present study the age range of patients was between 28 to 80 years, with majority of patients in age group of 50 to 59 years. The mean age of presentation of histological subtypes was Infiltrating duct carcinoma , no special type (IDC-NST) – 53.56 years. Infiltrating lobular carcinoma (ILC) – 54.08 years. Other histological subtypes – 57.07 years. This distribution was not significant statistically. (p=0.558, F=0.585, AVOVA.) The mean age of presentation of molecular subtypes was Luminal A – 60.06 years. Luminal B Her2 negative – 55.26 years. Luminal B Her2 amplified – 51.41 years. Triple negative Breast cancer (TNBC) – 49.76 years HER2 type – 52.17 years. This distribution showed a significant statistical difference when analyzed by analysis of variance (ANOVA) with p value equal to 0.02. It was found that the mean age at presentation of TNBC is significantly lower as compared to other subtypes whereas Luminal A subtypes is more commonly seen in the older age group. The results obtained in this study were similar to few studies conducted in other populations[19]. In our study out of 278 patients, 105 were in premenopausal group and 172 were in postmenopausal group. The distribution of molecular subtypes was in this manner. In Luminal A 80.9 percent (55 of 68) were in postmenopausal group and 19.1 percent (13 of 68) were in premenopausal group. Among Luminal B HER2 negative, 68.4 percent (26 of 38) were in postmenopausal group and 31.6 percent (12 of 38) were in premenopausal group. Out of 59 Luminal B HER2 amplified, 52.5 percent (31) were postmenopausal and 47.5 percent (28) were premenopausal. Among 66 patients of TNBC subtype, 48.5 percent (32) were postmenopausal and 51.5 percent (34) were premenopausal. In HER2 subtype 61.7 percent (29 of 47) were postmenopausal and 38.3% (18 of 47) were premenopausal. Most of the studies conducted in other populations have provided similar results but some studies has been found to have contrasting results also. This may be due to difference in the genetic pool of the population where the study was conducted[20].In present study out of 31.3 percent (87 of 278) tumours had Ki67 less than 14 percent and 68.7 percent (191 of 278) tumours had Ki67 more than 14 percentKi67 is incorporated as an important criterion to differentiate Luminal A from Luminal B Her2 negative tumours. In tumours which are positive for ER and /or PR and are negative for HER2, the Ki67 index is evaluated. Those with Ki67 >14 percent are classified as Luminal B HER2 negative and those with Ki67 <14 percent are classified as Luminal A. We analyzed both the histological subtypes and the molecular subtypes with two categories of Ki67 (<14% and >14%).By Pearson Chi square test there was no significant statistical association between histological subtypes and Ki67 index (p=0.08)All Luminal A tumours (100%) had Ki67 less than 14 percent and all Luminal B Her2 negative tumours (100%) had Ki67 greater than 14 percent. Among Luminal B Her2 amplified 81.4 percent (48) had Ki67 greater than 14 percent and 18.6 percent (11) had Ki67 less than 14 percent. Most of the TNBC patients, 93.9 percent (62 of 66) had Ki67 greater than 14 percent and rest had Ki67 less than 14 percent (6.1%, 4 of 66). Among HER2 subtype 91.5 percent (43 of 47) tumours had Ki67 more than 14 percent and 8.5 percent (4 of 47) had Ki67 less than 14 percent. This distribution had a significant p value of 0.004 by Pearson Chi square test. The results of the present study are in accordance with the results of studies conducted in other populations[21,22]

Conclusion

In comparison to histological subtypes, molecular subtypes can be a better tool for analysing the behaviour and disease aggressiveness of breast cancer, according to the findings of this study. We recommend that molecular classification be performed on all breast cancers and that it be used in conjunction with histological classification. It would be premature to dismiss histological classification at this time.

References

1. Kumar P, Bolshette NB, Jamdade VS, Mundhe NA, Thakur KK, Saikia KK, et al. Breast cancer status in India: An overview. Biomed Prev Nutr. 2013; 3(2):177-183.
2. Murthy NS, Chaudhry K, Nadayil D, Agarwal UK, Saxena S. Changing trends in incidence of breast cancer: Indian scenario. India J cancer 2009; 46(1):73-4.
3. Shawarby MA, Al-Tamimi DM, Ahmed A. Molecular classification of breast cancer: An overview with emphasis on ethnic variations and future perspectives. Saudi J Med MedSci 2013; 1(1):14-19.
4. Spitale A, Mazzola P, Soldini D, Mazzucchelli L, BordoniA. Breast cancer classification according to immunohistochemical markers: clinicopathologic features and short-term survival analysis in a population-based study from the South of Switzerland. Ann Oncol. 2009; 20(4):628-635.
5. Perou CM. Molecular stratification of triple-negative breast cancers. Oncologist. 2010; 15(5):39-48.
6. O'Shaughnessy J, Osborne C, Pippen JE, Yoffe M, Patt D, Rocha C, et al. Iniparib plus chemotherapy in metastatic triple-negative breast cancer. N Engl J Med. 2011; 364(3):205-14.
7. Perou CM, Sorlie T, Eisen MB, van de Rijin M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000 ; 406(6797):747-752.
8. Cleator S, Ashworth A. Molecular profiling of breast cancer: Clinical implications. Br J Cancer 2004; 90:1120-1124.
9. Sotiriou C, Neo SY, McShane LM, Korn EL, Long PM, Jazaeri A, et al. Breast cancer classification and prognosis based on gene expression profiles from a populationbased study. Proc Natl Acad Sci USA 2003; 100(18):10393-10398.
10. Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, HuZ, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma.Clin Cancer Res 2004; 10(16):5367-5374.
11. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, JohnsenH,et al.Gene expression patterns of breast carcinomas distinguish tumour subclasses with clinical implications. Proc Natl Acad Sci 2001; 98(19):10869-10874.
12. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thuürlimann B, Senn HJ, et al. Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer. Ann Oncol 2011; 22(8):1736–1747.
13. Tamimi RM, Baer HJ, Marotti J, Galan M, Galaburda L, Fu Y, et Comparison of molecular phenotypes of ductal carcinoma in situ and invasive breast cancer. Breast Cancer Res.2008; 10(4):R67.
14. Clark SE, Warwick J, Carpenter R, Bowen RL, Duffy SW, Jones JL. Molecular subtyping of DCIS: heterogeneity of breast cancer

reflected in pre-invasive disease.Br J Cancer 2011; 104(1):120-127.
15. Brenton JD, Carey LA, Ahmed AA, Caldas C: Molecular classification and molecular forecasting of breast cancer: ready for clinical application? J ClinOncol 2005; 23(29):7350-7360.
16. Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Research 2010; 12(5): R68.
17. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med. 2004; 351(27):2817-26.
18. Esteva FJ, Sahin AA, Cristofanilli M, Coombes K, Lee SJ, Baker J, et al. Prognostic role of a multigene reverse transcriptase-PCR assay in patients with node-negative breast cancer not receiving adjuvant systemic therapy. Clin Cancer Res. 2005; 11(9):3315-9.
19. Mina L, Soule SE, Badve S, Baehner FL, Baker J, Cronin M, et al. Predicting response to primary chemotherapy:gene expression profiling of paraffin-embedded core biopsy tissue. Breast Cancer Res Treat. 2007; 103(2):197-208. Epub 2006 Oct 13.
20. Geradts J, Bean SM, Bentley RC, Barry WT. The oncotype DX recurrence score is correlated with a composite index including routinely reported pathobiologic features. Cancer Invest. 2010;28(9):969-77.
21. van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature. 2002; 415(6871):530-6.
22. Sotiriou C, Wirapati P, Loi S, Harris A, Fox S, Smeds J, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. J Natl Cancer Inst. 2006 ; 98(4):262-72.

Conflict of Interest: Nil
Source of support:Nil