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Molecular Study Of Breast Cancer By Immunohistochemistry And Its Correlation With Histopathological Grading

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Abstract

Breast cancer is the most common cancer in women. An observational analytical study was carried out on 100 modified radical mastectomy specimens and the expression of ER, PR, HER2/neu and Ki 67 were studied and cases were classified as Luminal A, Luminal B, HER2/neu Enriched and Triple negative and then correlated with histopathological grading done according to Modified Scarff Bloom Richardson Grading. Out of 100 cases most common molecular subtype was Luminal A (40%), followed by Triple negative (34%), Luminal B (21%), HER2/neu enriched (5%). Most of Luminal A tumors showed low proliferation index whereas Triple negative and HER2/neu enriched tumors showed high proliferation index. When correlated with histopathological grading, most of Luminal A carcinoma cases were low grade whereas Triple Negative and HER2/neu enriched cases were high grade and were associated with higher TNM staging. Desmoplasia and Lymphovascular invasion traditionally being associated with bad prognosis were positively associated with higher stages. Also, large tumor size and lymph node metastasis were associated with poor prognosis. Correlation of the molecular classification with histopathological grade and with Ki67 was statistically significant (p value<0.0001%) which shows that molecular subtypes are directly related to the prognosis and thus guide the oncologists for planning the treatment. Also, tumor size, lymph node metastasis, lymphovascular invasion and desmoplasia serve as prognostic markers.

Keywords: Breast Cancer, Molecular Classification

Introduction

Breast cancer is a heterogenous disease resulting from many genetic and epigenetic events. Breast cancer has been classified traditionally based on morphology according to histopathological grading. But it was observed that patients having identical histopathological tumors had different prognosis which could be due to molecular differences. Also advances in the treatment focusing more on breast conservation has led to consideration of molecular subtypes. Immunohistochemistry was developed more than 25 years ago and it currently forms the cornerstone of molecular classification of breast carcinoma into ER +ve and ER –ve categories. [(1)] Along with the traditional molecular markers ER, PR and HER2/neu, recently Ki 67, the marker of proliferation is also being increasingly used to predict the prognosis much more accurately.

The traditional histomorphological classification of breast cancer based on anatomical and histological

properties of the tumor is not able to evaluate biological and molecular heterogeneity. Therefore, molecular classification should be considered for clinical management. Different markers respond differently to therapies. Luminal groups respond well to hormonal therapies, HER2/neu enriched tumors respond well to trastuzumab while basal like respond to chemotherapy. While genetic testing is costly and not available widely, therefore, at present, immunohistochemistry is accepted as an adequate surrogate marker as it is not that expensive and nowadays is becoming more popular and easily available. [(2)]

This study was undertaken to give an insight on the role of these molecular subtypes in deciding the prognosis and treatment plan of the patient thereby playing an important role as a diagnostic oncologist apart from being just a pathologist.

MATERIALS AND METHODS

An observational analytical study was carried out on 100 samples in the Department of Pathology, of our Medical College & Hospital, from June 2019 to June 2020. Gross examination, sampling and processing of the modified radical mastectomy specimens were done as per the standard protocols. H & E staining was done for histopathological examination. Histopathological grading was done according to Modified Scarff Bloom Richardson Scoring. Immunohistochemistry was performed. Known breast tissue positive for ER, PR and Her 2/Neu and proliferating follicles in a reactive lymph node were taken as positive controls.

The immunostained slides were be examined for- **NUCLEAR STAINING** for ER, PR and Ki 67 and **MEMBRANE STAINING** for Her2/neu.

Allred score method is used for hormone receptors considering the proportion of positive staining tumor cells along with the average intensity of staining**.** ER/PR staining is scored from 0-5. HER2/neu proteins are receptors on breast cells which normally control healthy breast cell growth, division, and repair. In HER2/neu gene amplification, HER2/neu gene makes multiple copies leading to uncontrolled growth of breast tissue. HER2/neu staining is scored from 0-3+. Ki-67 is a nuclear protein encoded by MK167 gene and is a most reliable indicators of proliferative activity of breast cancer. It is scored as percentage of positively stained cells among the total number of malignant cells and divided into two groups-low (<15%) and high (>15%).

St. Gallen has classified breast carcinoma into a 5-tyre system [(3)]. **Luminal A** (ER/PR+ve, HER2/neu-ve, low proliferation rate) **Luminal B HER2/neu Negative** (ER +ve /PR variable HER2/neu negative, Higher proliferation rate) **Luminal B HER2/neu Positive** (ER +ve /PR variable, HER2/neu positive, Higher proliferation rate), **Her2/neu Non-Luminal** (ER -ve /PR -ve, HER2/neu positive, **Basal Like/Triple Negative** (ER -ve /PR -ve, HER2/neu negative).The expression of ER, PR, HER2/neu and Ki67 index were studied and based on their expression were classified into Luminal A, Luminal B, HER2/neu enriched and Basal type. The molecular subtypes were then correlated with the histopathological grading.

INCLUSION CRITERIA:

100 mastectomy specimens from patients of all age groups diagnosed with breast carcinoma were included in the study.

EXCLUSION CRITERIA:

1. Patients with previous lumpectomy.
2. Patients who underwent neoadjuvant chemotherapy.
3. Patients with recurrence.

Data entry and analysis were done in Excel sheet version 2016 and SPSS version 25. Results were calculated and displayed in the form of proportions and percentages. The statistical analysis for correlation among these parameters were determined using the Pearson Chi square test/ Fisher Exact Test. Significance to be assumed at P VALUE <0.05.

This research has been forwarded by the Institutional Ethics Committee.

RESULTS

The present study comprises of 100 cases of breast carcinoma. Age of the patients ranged from 28-80 years. 40 % patients belonged to age group of 41-50 years with mean age of 44 years with only 1 male case. Left breast was more frequently involved (52%) as compared to right breast with only 1 % case showing bilateral involvement. Most of the patients belonged to postmenopausal age group (45.45%).

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More than half of the tumors were falling in the size range of 2-5 cm (59%), followed by 29% of tumors having size >5 cm and only 12% tumors had size less than 2 cm.

53% of cases belonged to grade II Category whereas 37% cases showed grade III features and only 10% cases were grade I. Maximum number of cases of grade II tumor (54.72%) belonged to age group of 41-50 years whereas grade III tumors showed bimodal peak at age groups 31-40 years (27.03%) and 51-60 years (37.83%).11% of tumors belonged to stage I, 54% cases belonged to stage II,33% cases stage III and 2% cases stage IV. Only 2% cases showed distant metastasis. 95.65% cases of stage II were in the age range of 41-60 years.

Maximum number of cases were Luminal A (40%) followed by Basal like (34%) and Luminal B (21%) and least number of cases belonged to HER2/neu enriched subtype (5%). Out of 21 cases of Luminal B subtype, 23.81% cases were positive for both ER/PR, 76.19% cases were positive for ER but negative for PR.9.52% cases showed HER2/neu positivity and belonged to Luminal B HER2/neu positive group whereas 90.48% cases were Luminal B HER2/neu negative.

Lymph node metastasis was present in 41% cases whereas 59% tumors were free from nodal metastasis. Only 13.11% of ER positive cases showed LN metastasis whereas 84.61% ER negative cases showed LN metastasis. (p value<0.001*)

Maximum lymph node metastasis was seen in Basal Like subtype (70.73%) whereas only 7.32% of Luminal A cases showed lymph node metastasis (p value<0.001*). Basal like subtype of breast carcinoma were associated with higher tumor size whereas Luminal A was associated with smaller tumor size (p value<0.001*). Luminal A subtype was associated with lower grade tumor and Basal Like and HER2/neu enriched subtype were associated with higher grade tumors (p value<0.001*). Luminal subtypes were associated with lower TNM stages whereas Basal Like subtypes were associated with higher TNM stages. (p value <0.001*)

82.5 % of Luminal A tumors showed low proliferation index whereas 79.41% of Basal Like and 60% of HER2/neu subtypes showed high proliferation index (p value<0.001*).

Only 24% cases showed Lymphovascular invasion.100% of stage IV showed LVI, followed by 42.42% cases of Stage III and 12.96% of Stage II. Only 9.09% of Stage I showed LVI. (p value<0.001*)80% of HER2/neu enriched cases showed lymphovascular invasion, followed by Luminal B (38.09%) and Basal Like (29.41%) (p value <0.0002). No significant correlation was found between LN metastasis and LVI (p value 0.43).

Desmoplastic reaction was present in 42% cases. 100% cases of stage IV showed desmoplasia followed by stage III (63.64%), stage II and stage I. (p value- 0.0027) 64.28% cases which were positive for LN metastasis showed desmoplastic reaction as well (p value-0.064).

Most common types of breast carcinoma was invasive ductal carcinoma, not otherwise specified (IDC-NOS) (78%). IDC with squamoid differentiation, Medullary Carcinoma, Secretory Carcinoma, IDC with Paget's disease of nipple and 41.03% cases of IDC-NOS were ER, PR Negative, whereas all the cases of ILC, invasive lobular carcinoma with Ductal carcinoma In situ and Lobular Carcinoma In situ, invasive ductal carcinoma with invasive lobular carcinoma, IDC with neuroendocrine differentiation, Mucinous Carcinoma, Solid papillary carcinoma, Papillary DCIS, Tubular carcinoma, 38.46% cases of IDC-NOS were ER/PR positive.

20.51 % cases of IDC-NOS were positive for ER but negative for PR. Only 6.41% cases of IDC-NOS showed HER2/neu positivity and 100% cases of IDC with Paget's disease of nipple showed positive HER2/neu status. All the medullary carcinoma cases were negative for HER2/neu, i.e. all the medullary carcinoma cases were triple negative.

DISCUSSION

In the present study, the peak age group was from 40-50 years of age, followed by 51-60 years of age which was consistent with an Indian study [4].

Majority of women in the present study were postmenopausal (45.45%). Another study [5] had 85.7% postmenopausal women in her study.

Present study has only 1 male patient. Male breast cancer is a rare disease with an incidence of 0.5-1% worldwide.[6] Left breast was involved by the tumor

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more frequently compared to right breast. However, side involved by the tumor does not proved to be significant in this study which is similar to the Indian study [4]. In 59% cases tumor was in the size range of 2-5 cm which is similar to other studies. [7,8]

Luminal A was the most common molecular subtype (40%) followed by basal like (34%), luminal B (21%) and Her2/neu enriched (5%) which is comparable with other Indian and foreign studies[8,9].

In the present study, ER positive tumors had negative lymph node metastasis (p<0.001*). In one study [10] ER Beta positivity was associated with negative axillary node status (p value<0.0001)

In the present study, no case presented with ER negative/ PR positive tumor consistent with another study [11]. But a few studies [12,13] showed ER negative/PR positive tumors. However, ER negative/ PR positive cases were reevaluated in India [14] and they concluded that high incidence of ER negative/ PR positive cases of breast carcinoma that are reported from India is most likely due to the use of suboptimal manual assays. When IHC was repeated all ER negative tumors turned out to be ER positive. A study [15] concluded that ER negative/PR positive breast cancer is not a reproducible subtype and PR testing is of uncertain clinical utility in ER negative breast cancer. Therefore, currently there is no strong evidence to support any clinical utility of PR positivity in ER negative tumors.

In the present study, statistically significant correlation was found between ER, PR, HER2/neu enriched and basal like with various histological grades of breast carcinoma with a p value <0.001. Most of the grade III tumors were HER2/neu enriched or Basal like, whereas most of the Luminal A tumors were low grade. Also, the molecular subtypes are significantly associated with TNM staging with p value <0.001. Luminal A belonged to lower TNM stages (Stage I and stage II) whereas HER2/neu enriched and Basal Like belonged to higher TNM stage (Stage III and stage IV) which is similar to other study with p value 0.016. [4] Also Basal like tumors were significantly associated with greater tumor size (p value - <0.001*) showing similarity with the Indian study [16] as well as higher lymph node metastasis. (p value <0.001*) consistent with an Indian study [4] showing similar results.

A high Ki 67 index (>/=15%) was significantly correlated with HER2/neu enriched and Basal like subtype whereas low Ki 67 index (</=15%) was significantly correlated with Luminal A subtype. Both these findings had p value <0.001. Statistically significant correlation was also found in many other Indian studies [17], [18], [19] and a few international ones. [20],[21]

Ki 67 has been confirmed as a prognostic factor in breast cancer patients in a routine setting. [22]

In the present study, significant relationship of lymphovascular invasion was seen with increasing stages of breast carcinoma (p value-<0.001*). Both the cases of Stage IV (100%) showed presence of lymphovascular invasion (LVI) followed by Stage III (42.42%), Stage II (12.96%) and least in Stage I (9.09%) which is consistent with the other study. [23]

An American study showed lymphovascular invasion in breast cancer subtypes as an important prognostic factor for recurrence free survival but there was no significant association between lymphovascular invasion and lymph node status. In the present study also lymphovascular invasion was significantly associated with HER2/neu enriched and Luminal B subtypes (p value-0.002*) [24] but no significant association was seen between lymphovascular invasion and lymph node status (p value-0.43). A study done in 2011 elaborated on the presence of LVI in LN negative tumors and concluded that the prognostic value of LVI was equivalent to that of 1-3 positive lymph nodes if the tumor was LVI negative[25]. Also patients with LVI who are negative for lymph node metastasis were recommended for neoadjuvant therapy. [26]

In the present study, desmoplastic reaction was significantly associated with higher staging of breast cancer i.e. maximum in stage III, followed by stage II (p value-0.0027). Paget's 'Seed and Soil' hypothesis in 1889, described active role of tumor stroma in the growth and spread of neoplastic cells [27]. At in situ carcinoma stage, growth factors like PDGF [28] or TGF-β [29] stimulate myofibroblasts within the adjacent stroma resulting in secretion of various chemokines, cytokines, growth factors and inflammatory mediators which contribute in oncogenesis. Myofibroblasts also produce collagen and extracellular matrix proteins which forms the 'desmoplastic stroma'. The stromal desmoplastic

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reaction was classified histologically into three categories, mature, intermediate and immature and was observed that immature stroma is associated with invasion and therefore resulting in poor prognosis and increased recurrences. [30]It was observed that an increase in percentage of lymph node metastasis when desmoplastic reaction transformed from mature to intermediate to immature and concluded that desmoplastic reaction in the stroma can be potentially utilized to predict grade and nodal status of patient. [31]. However, in this study, no significant correlation was found between desmoplastic reaction and lymph node metastasis. (p value- 0.064)

CONCLUSION

This study shows that the molecular subtypes and histological subtypes are significantly correlated and tumor size, lymph node metastasis and Ki67 index are known to affect the prognosis and they reflect that Luminal A and Luminal B belong to a group with better prognosis had better prognosis as compared to basal like and HER2/neu enriched.Luminal groups tend to have a lower proliferative index suggesting better prognosis while HER2/neu enriched and Basal like have higher proliferative index therefore reflecting poor prognosis.This study highlights that molecular classification is important as few grade III tumors belong to Luminal A and Luminal B subtypes and a few grade II tumors were Basal Like. This means that although maximum number of cases correlated but few cases being moderately to poorly differentiated in histopathological grading actually belonged to molecular groups with better prognosis as they can respond to targeted therapies and vice versa. Therefore, with the increasing use of molecular techniques and better availability of newer drugs focusing more towards targeted therapies, molecular classification of breast cancer is proposed for prognosis, treatment and outcomes.

TABLE 1: Relationship Of ER Positivity, Lymphovascular Invasion (LVI) And Desmoplasia With LN Metastasis.

	LN METASTASIS PRESENT	LN METASTASIS ABSENT	P VALUE
ER+ve/PR+ve	4	41	<0.001*
ER+ve/PR-ve	4	12	
ER-ve/PR-ve	33	6	
LVI PRESENT	18 (43.91%)	6 (10.17%)	0.43
LVI ABSENT	23 (56.09%)	53 (89.83%)	
DESMOPLASIA PRESENT	27 (64.28%)	15 (35.72%)	0.064

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TABLE 2: Relationship Of Lymphovascular Invasion (LVI) With TNM Staging

	STAGE I	STAGE II	STAGE III	STAGE IV	P value
LYMPHO VASCULAR INASION PRESENT	1 (9.09%)	7 (12.96%)	14 (42.42%)	2 (100%)	
LYMPHO VASCULAR INVASION ABSENT	10 (90.91%)	47 (87.04%)	19 (57.58%)	0	<0.001*
DESMOPLASIA PRESENT	1 (9.09%)	20 (37.04%)	21 (63.64%)	0	
DESMOPLASIA ABSENT	10 (90.91%)	34 (62.96%)	12 (36.36%)	2 (100%)	0.0027

TABLE 3: Clinicopathological And Immunohistochemical Characteristics Of Breast Cancer Cases

VARIABLES		ALL CASES	LUMINAL A	LUMINAL B	HER2/neu POSITIVE	BASAL LIKE	P VALUE
AGE GROUP	**21-50 Years**	54	17	17	3	17	
	51-80 Years	46	23	4	2	17	0.036
TUMOR SIZE	**<2 cm**	12	10	2	0	0	
	2-5 cm	59	30	15	2	12	<0.001*
	>5 cm	29	0	4	3	22	
GRADING	**GRADE I**	10	10	0	0	0	
	GRADE II	53	26	16	0	11	<0.001*
	GRADE III	37	4	5	5	23	
LN METASTASIS	**PRESENT**	41	3	5	4	29	
	ABSENT	59	37	16	1	5	<0.001*
TNM STAGE	**STAGE I**	11	9	2	0	0	
	STAGE II	54	30	14	2	8	
	STAGE III	33	1	5	3	24	<0.001*
	STAGE IV	2	0	0	0	2	
	HIGH	48	33	6	2	7	

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Ki 67	**LOW**	52	7	15	3	27	<0.001*
LYMPHO VASCULAR INVASION	**PRESENT**	24	2	8	4	10	<0.0002
	ABSENT	76	38	13	1	24	

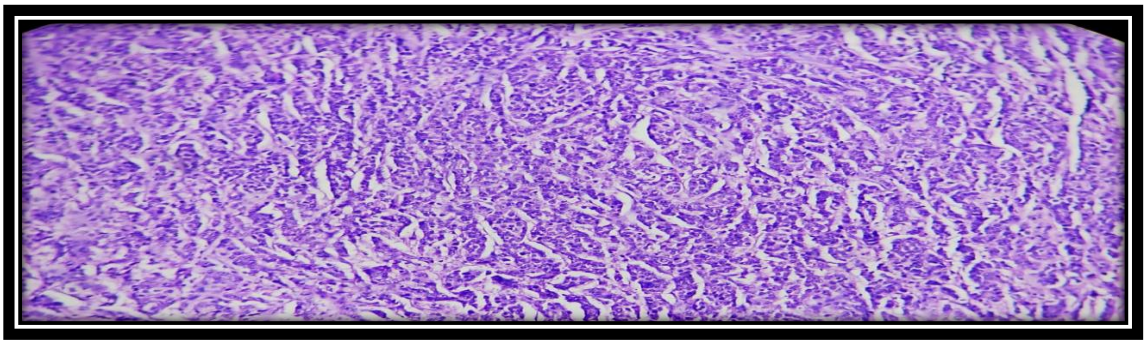


FIG. 1- GRADE III- BREAST CARCINOMA (10X)- Showing absence of tubule formation

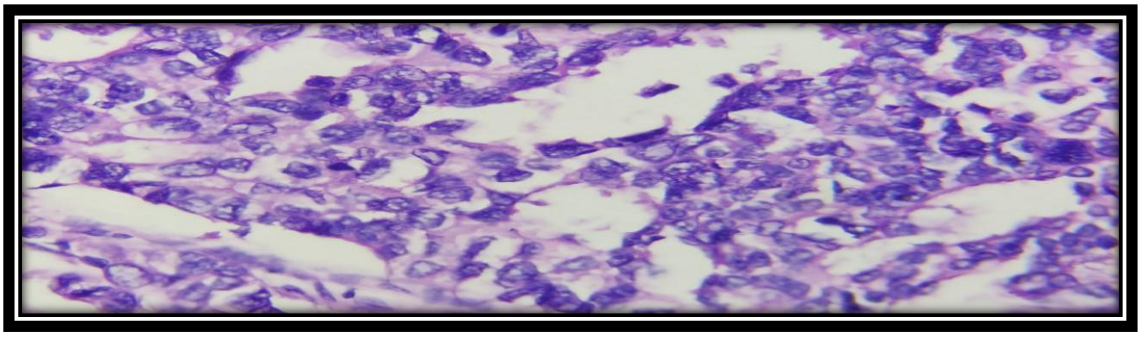
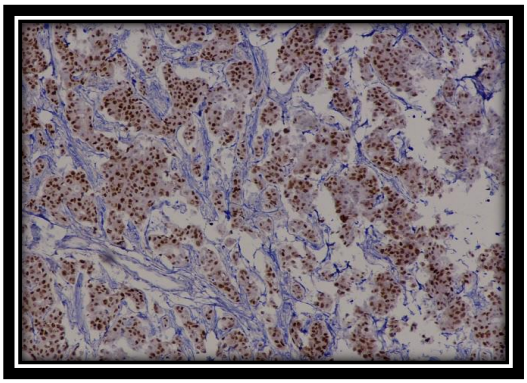


FIG. 2 - GRADE III- BREAST CARCINOMA (40X)-Showing marked nuclear pleomorphism and high mitotic count. Arrow indicating mitosis.



Figures From The Same Patient Depicting Grade III Breast Carcinoma Histopathologically But Showing ER And PR Positivity.

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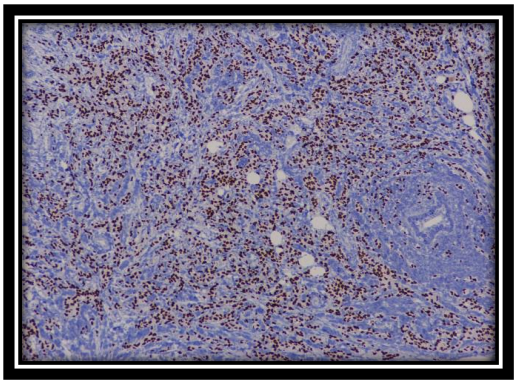

REFERENCES

1. Lajos Pusztai, Chafika Mazouni, Keith Anderson,Yun Wu, W.Fraser Symmans; Molecular classification of breast cancer : Limitations and potential. The Oncologist September 2006 vol11no.8(868-877)
2. Arnis Abolins; Molecular subtypes and immunohistochemical profiles in breast cancer. Riga Stradins University.Dissertation;2012
3. Vasconcelos I, Hussainzada A, Berger S, Fietze E, Linke J, Siedentopf F, Schoenegg W. The St. Gallen surrogate classification for breast cancer subtypes successfully predicts tumor presenting features, nodal involvement, recurrence patterns and disease-free survival. The Breast.2016 Oct;29:181-5. doi: 10.1016/j.breast.2016.07.016. Epub 2016 Aug 18
4. Smriti Tiwari ,Reeni Malik, VK Trichal, RK Nigam, Arvind Rai, Sharda Balani, Jaya Jain, Dhruvendra Pandey. Breast Cancer: Correlation of Molecular Classification with Clinicohistopathology Sch. J. App. Med. Sci., 2015; 3(2G):1018-1026
5. F.DeWaard , E.A. Baan ders Van Halewijn, J.Huizinga. The bimodal age distributionofpatients with mammary carcinoma. Evidence for the existence of 2 types of human breast cancer. Cancer 1964;(17):141-151.
6. Deepak Sundriyal,Sumedha Kotwal, Ramesh Dawar, and K. M. Parthasarathy.Male Breast Cancer in India: Series from a Cancer Research CentreIndian J Surg Oncol. 2015 Dec; 6(4): 384–386.Published online 2015 Oct 19. doi: 10.1007/s13193-015-0473-1.
7. Lakmini K.B. Mudduwa.Quick score of hormone receptor status of breast carcinoma: correlation with the other clinicopathologic parameters . Indian Journal of Pathology and Microbiology 2009;52(2):159-163.
8. Nikhilesh Kumar, Brig, Preeti Patni, Lt Col, A. Agarwal, Lt Col, M.A. Khan, Col, and Nidhi Parashar; Prevalence of molecular subtypes of invasive breast cancer: A retrospective study, 2015 Jul; 71(3): 254–258.
9. Adedayo A. Onitilo, Jessica M.Engel, Robert T. Greenlee, Bickol N. Mukesh. Breast Cancer Subtypes based on ER/PR and HER2 expression : Comparison of Clinicopathologic Features and Survival. Clinical Medicine & Research 2009;7:4-13.
10. Tero A.H. Jarviner, Markku Petto-Huikko, Kaija Holli, Torma Isola.Estrogen receptor beta is coexpressed ER alpha and PR and associated with nodal status, grade and proliferation rate in breast cancer. Am J Pathol2000;156(1):29-35.
11. Mehrdad Nadji , Carmen Gomez Fernandez, Parvin Ganjei Azar, AzoridesR.Moarales. Immunohistochemistry of estrogen and progesterone receptors reconsidered: Experience with 5993 breast cancers. Am J Clin Pathol2005;123:21-27.
12. Shiv Pankaj Khanna[1], Rigvardhan[2]. Correlation of Histological Grade with Hormone Receptors and Her 2 Neu In Carcinoma Breast - A Tertiary Centre Study.

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International Journal of Contemporary Medical Research Volume 5 | Issue 5 | May 2018 | ICV: 77.83 | ISSN (Online): 2393-915X; (Print): 2454-7379.

13. Inwald EC, Klinkhammer-Schalke M, Hofstädter F: Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry. Breast Cancer Res Treat 139(2):539-52, 2013.
14. Navani S, Bhaduri AS. High incidence of estrogen receptor negative progesterone receptor positive phenotype in Indian breast cancer: fact or fiction ? Indian J Pathol Microbiol 2005;48(2):199-201.
15. Hefti, M. M., Hu, R., Knoblauch, N. W., Collins, L. C., Haibe-Kains, B., Tamimi, R. M. & Beck, A. H. (2013). Estrogen receptor negative/progesterone receptor positive breast cancer is not a reproducible subtype. Breast cancer research: BCR, 15(4), R68. PMID: 23971947.
16. Munjal K, Ambaye A, Evans MF, Mitchell J, Nandedkar S, Cooper K; Immunohistochemical Analysis of ER, PR, Her2 and CK5/6 in Infiltrative Breast Carcinomas in Indian Patients. Asian Pacific Journal of Cancer Prevention, 2009; 10(5). 773–778.
17. Leong SP, Shen ZZ, Liu TJ, Agarwal G, Tajima T, Paik NS, Sandelin K, Derossis A, Cody H,FoulkesWD;Is breast cancer the same disease in Asian and Western countries?;World J Surg. 2010Oct;34(10):2308-24. doi: 10.1007/s00268-010-0683-1
18. Shabnam Karangadan**,** Anuradha Ganesh Patil**,** Sainath Karnappa Andola **;**Immunohistochemical characterization of molecular classification of breast carcinoma and its relation with Ki-67,2016 ;5(5): 430-436.
19. Singhai R, Patil V, Patil A. Status of HER-2/neu receptors and Ki-67 in breast cancer of Indian women. Int J Appl Basic Med Res 2011;1:15-9.
20. Dang M, Mysorekar V. Correlation of the Expression of Estrogen Receptor, Progesterone Receptor, HER2/neu and Ki-67 with Clinical Features and Tumour Histopathology in Breast Carcinoma. RGUHS Dissertation; 2012.
21. Verma S, Bal A, Joshi K, Arora S, Singh G. Immunohistochemical characterization of molecular subtypes of invasive breast cancer: A study from North India. APMIS 2012;120:1008-19.
22. E C Inwald [1], M Klinkhammer-Schalke, F Hofstädter, F Zeman, M Koller, M Gerstenhauer, O Ortmann.Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry.Breast Cancer Res Treat. 2013 Jun;139(2):539-52.doi: 10.1007/s10549-013-2560-8. Epub 2013 May 16.
23. Nair GL,Tewari V. Prognostication and Correlation between Histopathology and IHC of Breast Cancer. RGUHS Dissertation;2011.
24. Guo-Shiou Liao1, Huan-Ming Hsu1, Chi-Hong Chu1, Zhi-Jie Hong1, Chun-Yu Fu1, Yu-Ching Chou2, Mehra Golshan3,Ming-Shen Dai4, Teng-Wei Chen1, Chan De-Chian1, Wan-Chen Tsai1, Chao-Wen Pan1, Kuo-Feng Hsu1, En-Nung Kao1,Yi-Chih Hsu5, Tsun-Hou Chang5, Jyh-Cherng Yu1Prognostic Role of Lymphovascular Invasion and Lymph Node Status among Breast Cancer Subtypes. J Med Sci 2018;38(2):54-61.DOI: 10.4103/jmedsci.jmedsci_105_17.
25. Rakha EA, Martin S, Lee AH, Morgan D, Pharoah PD, Hodi Z, *et al.* The prognostic significance of lymphovascular invasion in invasive breast carcinoma. Cancer2012;118:3670-80.
26. Jagsi R, Raad RA, Goldberg S, Sullivan T, Michaelson J, Powell SN,*et al.*Locoregional recurrence rates and prognostic factors for failure in node-negative patients treated with mastectomy: Implications for postmastectomy radiation. Int J Radiat Oncol Biol Phys 2005;62:1035-9.

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27. Mueller MM, Fusening NE. Friend or foes-bipolar effects of the tumor stroma in cancer. Nat Rev Cancer. 2004; 4(11): 839-849.

28. Shao Z-M, Nguyen M, Barsky SH: Human breast carcinoma desmoplasia is PDGF initiated. Oncogene 2000, 19:4337–4345.

29. Walker RA, Dearing SJ: Transforming growth factor b1 expression in in-situ and invasive breast cancers. Eur J Cancer 1992,28:641–644.

30. Ueno H, Jones AM, Wilkinson KH. Histological categorization of fibrotic cancer stroma in advanced rectal cancer. Gut. 2004; 53:581–586.

31. Mariam Shadan1, Nazoora Khan2, Mohammad Amanullah Khan2, Hena Ansari3, Sufian Zaheer4. Histological categorization of stromal desmoplasia in breast cancer and its diagnostic and prognostic utility. SSRG International Journal of Medical Science (SSRG-IJMS) – Volume 4 Issue 6 – June 2017

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