

Clinico-Cyto-Histopathological Correlation of Patterns Carcinoma Breast and Correlation of Grading to Prognostic Factor

ANKITA NARENDRA TAMHANE, KISHOR M HIWALE, SAMARTH AKHILESH SHUKLA

ABSTRACT

Introduction: Carcinoma breast is the most common malignancy occurring in females worldwide while in India it is the 2nd most common malignancy occurring after cervical cancer in females. The incidence is three times higher in urban areas than in rural setup. The disease pattern, clinical and histopathological presentation differs from that of the western world.

Aim: To study various patterns of carcinoma breast in rural setup, its clinical correlation with cytology and histopathological examination and correlation of nuclear grading with various prognostic markers.

Materials and Methods: The present prospective analytical study was carried out in the Department of Pathology, Jawaharlal Nehru Medical College and Acharya Vinoba Bhave Rural Hospital, Sawangi (M), Wardha, India, from July 2013 to July 2015 on 100 female patient cases of carcinoma breast calculated using confidence level. Cytosmears were stained by Giemsa, paraffin embedded tissue blocks of tumour masses

and lymphnodes by H & E. Tumour mass was further subjected to immunohistochemistry.

Results: Present study comprised of total 100 female patients. Out of all, 32% cases were found to be in 5th decade (premenopausal predilection) of life in our setup. About 44% patients had clinical Stage II. In 96.3% of Grade III tumours cytological and histopathological correlation was found. 92% of cases belonged to NOS category (Grade III). 41% Case belonged to Bloom- Richardson Grade III. Maximum lymphnode positivity with lymphovascular invasion was found in Grade III tumours. Positive correlation was found between clinical staging cytological grading, histology and immunohistochemistry.

Conclusion: From the present study it can be concluded that there is a positive correlation between histopathological grade and other prognostic factors including immunohistochemical markers. Immunohistochemical markers can be effectively used to predict prognosis and therapeutic management of patients with carcinoma breast.

Keywords: Bloom-Richardson grading, Lympho vascular invasion, Robinson grade

INTRODUCTION

Carcinoma breast is the most common malignancy occurring in females. Age Standardized Incidence Rate (ASR) is 39 per 1,00,000 which is more than double that of second rank malignancy (cervical cancer ASR=15.2 per 1,00,000) [1,2]. 23% of all newly occurring cancers in women worldwide is carcinoma breast and represents 13.7% of all cancer deaths [1]. In India, breast cancer second most common cancer (after cervical cancer). It is three times higher in urban areas than in rural parts of the country. The disease pattern and presentation of breast cancer stand out as compared to western countries. Premenopausal patients constitute 50% population in India as compared to west, average age of the patients is 50-53 years, patients most commonly present with Stage II disease, only 5% have family history, IDC (NOS) was found to be the most common type and 70% had Grade III disease [3]. Large number of patients had

poor prognostic histopathological sub-type inspite of clinical stage [4]. The aim of present study was to study various patterns of carcinoma breast in rural set-up, correlation of clinical stage with cytological and histopathological grade and also correlation of histopathological grade with various prognostic markers including molecular sub-type.

MATERIALS AND METHODS

The present prospective analytical study was carried out in the Department of Pathology, Jawaharlal Nehru Medical College and Acharya Vinoba Bhave Rural Hospital, Sawangi (M), Wardha, India from July 2013 to July 2015. Total 100 female were considered for the study, selected on the basis of inclusion and exclusion criteria. Cytosmears were stained with Giemsa stain, paraffin embedded tissue blocks of tumor masses and lymphnodes by haematoxylin and eosin. Tumor mass were subjected to immunohistochemistry. The above

study was approved by institutional ethical committee and informed consent was obtained from the patients prior to the study.

Inclusion criteria: Female patients in age group of 20-75 years with clinical suspicion of malignancy.

Exclusion criteria: Patients under 20 years with non neoplastic lesions. Immunohistochemistry (IHC) for Estrogen Receptor (ER), Progesterone Receptor (PR), HER-2/neu and Ki-67 was done by Dakophrm DX TM immunohistochemistry kit. Ductal lining of normal breast tissue was used as a positive control for ER and PR and previously positive cases for HER2/neu were taken as controls. Reactive lymphnode was taken as positive control for Ki-67. Major histopathological sub-types of invasive breast carcinoma are Invasive ductal carcinoma (Not otherwise specified), invasive lobular carcinoma, medullary carcinoma, colloid carcinoma, tubular carcinoma and papillary carcinoma.

Robinsons Grading for Cytology [5]: Six parameters were considered; cell dissociation, cell size, uniformity, nucleoli, nuclear margin, chromatin. Each is given a score of 1-3. Minimal score is 6 while maximum score is 18.

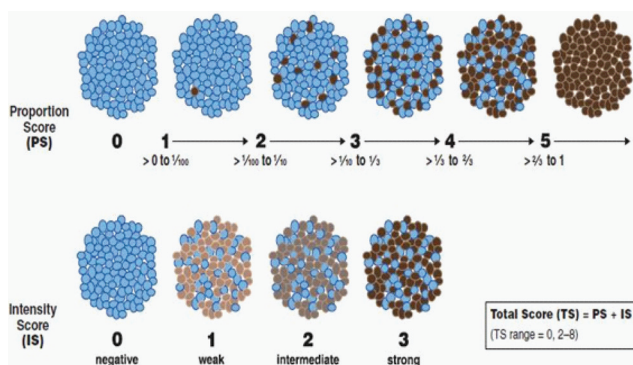
The Modified Scarff's Bloom-Richardson Histopathological Grading [6]: Tubule formation, nuclear pleomorphism and mitotic count (each given a count of 1-3).

Grade I (3-5), Grade II (6-7) and Grade III (8-9).

Total score : 0-2 Negative

3-8 Positive

Allred Scoring System [Table/Fig-1] [7]



[Table/Fig-1]: Calculation for Immunohistochemistry (Allred Scoring System) [7].

Grading for Immunohistochemical Staining of HER-2/neu Expression [8]: HER2/neu staining is graded from 0-3+; with no staining or membrane staining in more than 10% of tumor cells graded as 0 and strong complete membrane staining in more than 30% of tumor cells as 3+. 0-1 is negative; 2+,3+ is positive according to ASCO/CAP (American Society of Clinical Oncology and the College of American Pathologist).

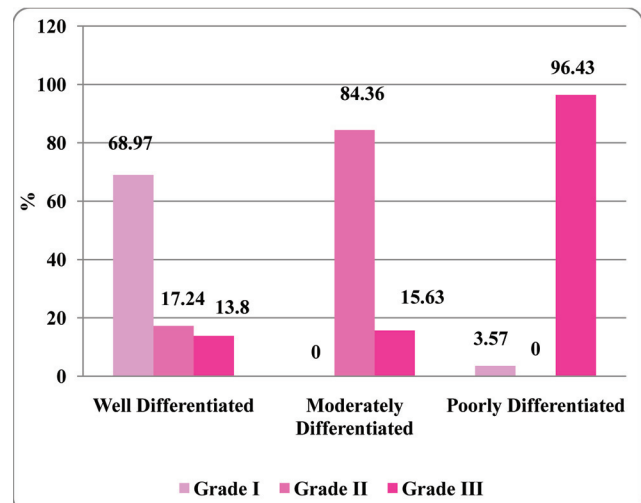
Ki-67 Proliferative Index [9]: Ki-67/MIB-1 antibody against proliferating nuclear antigen. Ki-67 positivity is seen as brown discoloration of the nuclei. It is calculated as

nuclear positivity in 100 cells per high power field and then the percentage is given. Less than 14% is negative while $\geq 14\%$ were positive.

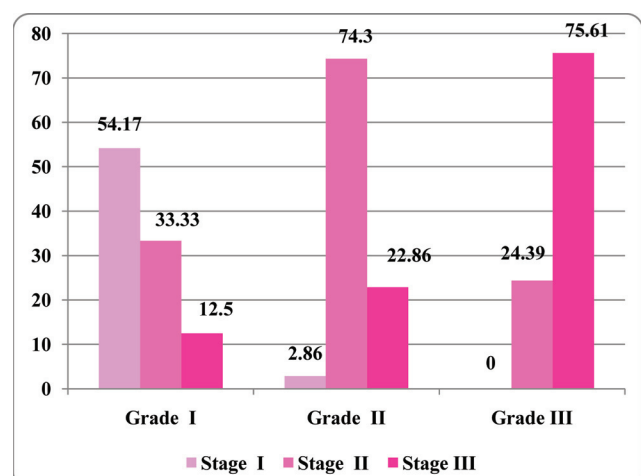
Distribution of cases is depicted by pie diagrams while correlations are depicted using multiple bar diagrams. Chi square test was used for calculating the significance for these correlations.

RESULTS

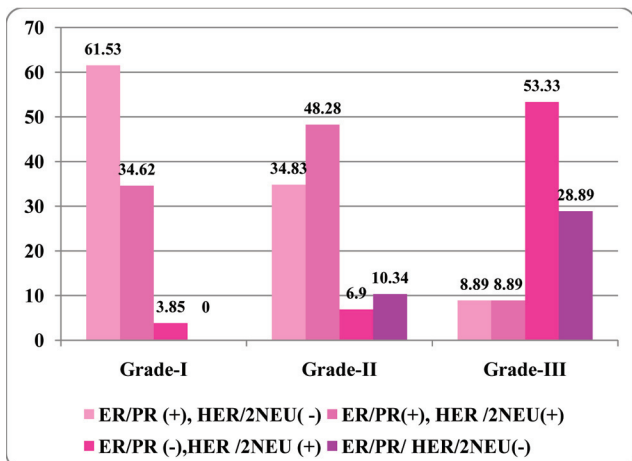
Present study comprised of 100 female patients. Out of 100, 32 cases were in 5th decade with premenopausal prevalence of the disease in our set up. About 44% cases were of clinical Stage II, about 96.3% Grade III cases cytological and histopathological correlation was found. 92% cases belonged to 'NOS' category, 41% cases belonged to Bloom- Richardson Grade III. Maximum lymphnode positivity with lymphovascular invasion was seen in Grade III tumors [Table/Fig-2-7].



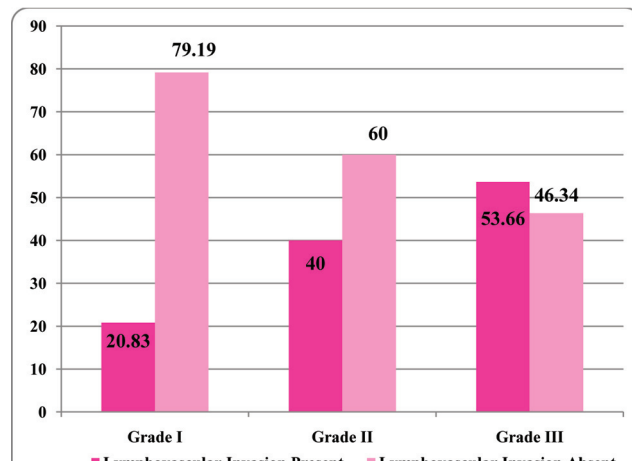
[Table/Fig-2]: Figure depicting correlation of cytological grade and histopathological grade.



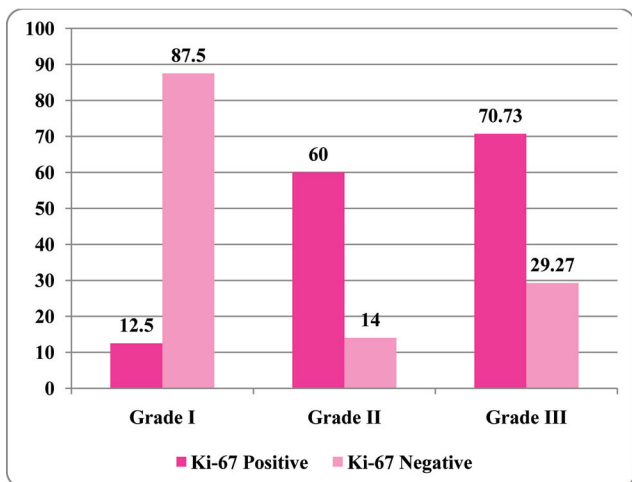
[Table/Fig-3]: Figure depicting correlation of clinical stage and histopathological grade.



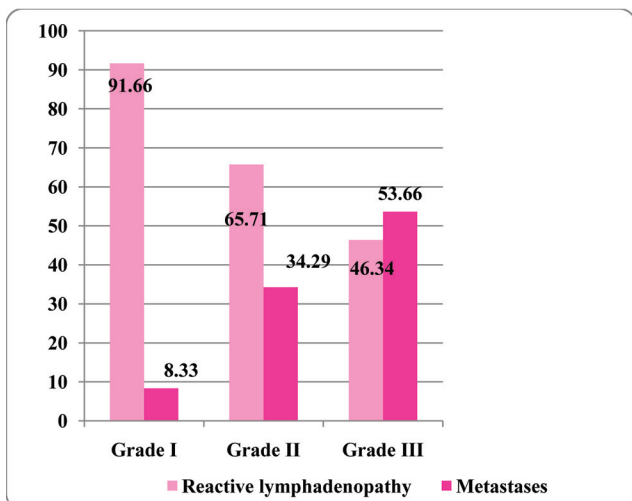
[Table/Fig-4]: Figure depicting correlation of histopathological grade and immunohistochemistry.



[Table/Fig-7]: Correlation of lymphovascular invasion and histopathological grade.



[Table/Fig-5]: Correlation of Ki-67 index and histopathological grade.



[Table/Fig-6]: Correlation of lymphnode metastases and histopathological grade.

DISCUSSION

It needs to be emphasized that the major utility of present study lies in correlating the clinical staging, cytological grading, immunohistochemical categorization and other histomorphological parameters with the histopathological grade in patients with carcinoma breast patients as markers of prognostic significance. It utilizes immunohistochemistry for prognostication and management of the patients with carcinoma breast.

Age wise Distribution of Patients: According to Agrawal et al., [10] the age standardized incidence for breast cancer in India is 22.9/1,00,000 which is 1/3rd that of western women and the mortality is disproportionately high in India [Table/Fig-8].

Sledge et al., (2005) [11]	Agarwal GW et al., (2007) [12]	Murthy NS et al., (2007) [13]	Mohammad HF et al., (2011) [14]	Present Study (2015)
50-53 years	Less than 50 years	Less than 45 years	Less than 50 years	41-50 years

[Table/Fig-8]: Age wise distribution of patients with carcinoma breast.

Distribution of histopathological patterns of cases: In the present study 94 cases (94%) cases IDC 'NOS' type comprised the most common pattern. Abeloff MD et al., [15] in their study on epidemiological factors observed IDC(NOS) as the most common presenting pattern of carcinoma breast. Stanley PL et al., [16] in their study on patterns of carcinoma breast found that IDC (NOS) was the most common pattern in Asian women [Table/Fig-9-11].

Fine needle aspiration cytology has proved a useful means for diagnosis of carcinoma breast patients presenting with obvious palpable lumps.

Correlation of Histological Grade with Immunohistochemical Markers: Gupta D et al., [28] studied 50 patients

Yang XR et al., [16]	El-Sayed ME et al., [17]	Syed BM et al., [18]	Quayson SE et al., [19]	Present Study
Positive correlation	Positive correlation	Positive correlation	Positive correlation	Positive correlation

[Table/Fig-9]: Correlation of clinical stage and histopathological grade.

Histo-logical Grade	Das S et al., (2013) [20]	Gore R et al., (2013) [21]	Rajan J et al., (2014) [22]	Einstien D et al., (2014) [23]	Ravi Kumar G et al., (2014) [24]	Present Study (2015)
Grade I	24	06	15	12	22	20
Grade II	42	38	20	40	61	27
Grade III	35	15	09	20	15	27
	101	59	44	72	98	74

[Table/Fig-10]: Correlation of cytological grade with histopathological grade.

Panjwani P et al., [25]	Keam B et al., [26]	Cubukcu E et al., [27]	Quayson SE et al., [19]	Present Study
Grade III	Grade III	Grade III	Grade III	Grade III

[Table/Fig-11]: Comparison of present study with other studies- histological grade wise distribution.

of carcinoma breast in which the hormonal receptor status was correlated with the histopathological grade and the lymphnode status. The observations were similar to the observations of our study that a reduction in ER/PR positivity is associated with increase in histopathological grade while increased lymphnode metastases is seen in these patients.

Mazouni C et al., [29] compared the biomarkers of carcinoma with lymphnode status that ER/PR (+) with HER2/neu (+) and only HER2/neu (+) category had a positive lymphnode metastases as compared to ER/PR (+) and HER2/neu (-) category.

A study conducted by Park S et al., [30] on 1006 operated breast cancer patients. They applied four biomarkers ie ER, PR, HER2/neu and Ki-67 to determine their significance in carcinoma breast patients. They concluded that ER/PR positivity with HER2/neu negativity is associated with a lower histopathological grade ie Grade I while only HER2/neu positive and triple negative breast cancers had grade III on histopathology.

A low grade tumor (Bloom-Richardson Grade I and II) showed higher ER and PR positivity than a high grade tumor which were more of ER/PR negative and HER2/neu positive or all three negative. Negative pattern of both of the hormonal (ER, PR) receptors with HER2/neu positivity is indicative of an aggressive disease.

Correlation of Lymphnode Status with Histopathological Grade: Azizun-Nisa YB et al., [31] in their study concluded that higher histopathological grade of tumor is associated

with an increase in HER2/neu expression and lowered ER/PR expression with increased incidence of lymphnode metastases.

Correlation of Histopathological Grade and Lymphovascular Invasion: Price GE et al., [32] found that metastasis of carcinoma breast is mainly through lymphovascular invasion. Song JU et al., [33] studied role of lymphovascular invasion as a positive prognostic marker for higher histopathological grade.

Correlation of Tumour Size with Histopathological Grade: Azizun-Nisa et al., [31] larger tumor size is associated with increased histopathological grade as was seen in the present study.

LIMITATIONS

1. Cost of immunohistochemistry is a concern.
2. Large lump due to severe desmoplasia was considered into clinical Stage III.
3. Cytological grading overlapped in few cases.

CONCLUSION

From the above study it can be concluded that there is a positive correlation between histopathological grade and other prognostic factors including immunohistochemical markers. Immunohistochemical markers can be effectively used to predict prognosis and therapeutic management of patients with carcinoma breast.

REFERENCES

- [1] Ferlay J, Bray F, Parkin DM, Pisani P, eds (2001) Globocan 2000: Cancer Incidence and Mortality Worldwide (IARC Cancer Bases No. 5), Lyon, IARC Press.
- [2] Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocon 2000. *Int J Cancer*. 2001;94:153-56.
- [3] Leong SP, Shen ZZ, Liu TJ, Agarwal G, Tajima T, Paik NS, et al. Is Breast Cancer the Same Disease in Asian and Western Countries? *World J Surg*. 2010;34(10):2308-24.
- [4] Desai SB, Moonim MT, Gill AK, Punia RS, Naresh KN, Chinoy RF. Hormone receptor status of breast cancer in India: a study of 798 tumors. *Breast*. 2000;9(5):267-70.
- [5] Comprehensive Cytopathology. Breast. In: Bibbo M, Wilbur DC. 4th ed. New Delhi: Reed Elsevier India Private Limited;2015. pp 583.
- [6] Rosai J. Breast. In: Rosai and Ackerman's Surgical Pathology. 9th ed. Noida: Reed Elsevier India Private limited. 2009.p.1787-827.
- [7] Fitzgibbons PL, Murphy DA, Hammond ME, Allred DC, Valenstein PN. Recommendations for validating estrogen and progesterone receptor immunohistochemistry assays. *Arch Pathol Lab Med*. 2010;134(6):930-35.
- [8] Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med*. 2007;131(1):18-43.
- [9] Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, et al. Ki67 index, HER2 status, and prognosis of patients with luminal

- B breast cancer. *J Natl Cancer Inst.* 2009;101(10):736-50.
- [10] Agarwal G, Ramakant P. Breast Cancer Care in India: The current scenario and the challenges for the future. *Breast Care (Basel)*. 2008;3(1):21-27.
- [11] Sledge GW. Cancer research in the developing world. 41st Annual Meeting of ASCO 2005. Educational book. 2005: 698–71.
- [12] Agarwal G, Pradeep PV, Aggarwal V, Yip CH, Cheung PS. Spectrum of breast cancer in Asian women. *World J Surg.* 2007;31(5):1031-40.
- [13] Murthy NS, Agarwal UK, Chaudhry K, Saxena S. A study on time trends in incidence of breast cancer –Indian scenario. *Eur J Cancer Care (Engl)*. 2007;16(2):185-86.
- [14] Mohammad HF, Foreman KJ, Delossantos AM, Lozano R, Lopez AD, Murray CJL, et al. Breast and cervical cancer in 187 countries between 1980 and 2010: a systematic analysis. *The Lancet*. 2011;378 (9801):1461-84.
- [15] Abeloff MD, Wolff AC, Weber BL, et al. *Cancer of the Breast*. Clinical Oncology. Philadelphia, Pa: Elsevier; 2008; 4:1875–1943.
- [16] Yang XR, Sherman ME, Rimm DL, Lissowska J, Brinton LA, Peplonska B, et al. Differences in risk factors for breast cancer molecular subtypes in a population-based study. *Cancer Epidemiol Biomarkers Prev.* 2007;16(3):439–43.
- [17] El-Sayed ME, Lee AH, Elston CW, Grainge MJ, Hodi Z, et al. Prognostic significance of nottingham histologic grade in invasive breast carcinoma. *J Clin Oncol.* 2008;26(19):3153-58.
- [18] Syed BM, Niaz A, Sangrasi AK, Susheel C, Talpur AK, et al. Breast cancer in Pakistan - a situation analysis. *Pak J Surg.* 2013;29(3):183-85.
- [19] Quayson SE, EK Wiredu, DN Adjei and Anim JT. Breast cancer in Accra, Ghana. *Journal of Medical and Biomedical Sciences.* 2014; 3(3):21-26.
- [20] Das S, Kalyani Kumar K. Breast carcinoma aspirates: A study of cytological grading. *JMS.* 2013;2:189-95.
- [21] Gore R, Shirish S, Aggrawal R, Vimal S, Deshpande H. Robinson cytological grading of breast carcinoma on fine needle aspiration cytology- an overview. *Int J Pharm Biol Sci.* 2013;3:564-70.
- [22] Rajan J and Pushpalatha Pai K. Robinson's cytological grading of breast carcinoma and its correlation with Scarff Bloom Richardson's histologic grading. *IJBR.* 2014;05(02).
- [23] Einstien D, Parijatham O, Ganapathy H, and Rahman S. Comparison of 3-Tier Cytological Grading Systems for Breast Carcinoma. *ISRN Oncology.* vol. 2014, Article ID 252103, 6 pages, 2014.
- [24] Ravikumar G, Rout P. Comparison of cytological versus histopathological grading of invasive ductal carcinoma of the breast with correlation of lymph node status. *Middle East Journal of Cancer.* 2015;6(2):91-96.
- [25] Panjwani P, Epari S, Karpate A, Shirsat H, Rajsekharan P, Basak R, et al. Assessment of HER2/neu status in breast cancer using fluorescence in situ hybridization and immunohistochemistry: Experience of a tertiary cancer referral centre in India. *Indian J Med Res.* 2010;132:287-294.
- [26] Keam B, Im SA, Lee KH, Han SW, Oh DY, Kim JH, et al. Ki-67 can be used for further classification of triple negative breast cancer into two subtypes with different response and prognosis. *Breast Cancer Research : BCR.* 2011;13(2):R22.
- [27] Cubukcu E, Kanat O, Fatih O, Kabul S, Canhoroz M, Avci N, et al. Prognostic significance of estrogen receptor, progesterone receptor, HER2/neu, Ki-67, and nm 23 expression in patients with invasive breast cancer. *J BUON.* 2013;18(2):359-65.
- [28] Gupta D, Gupta V, Marwah N, Gill M, Gupta S, Gupta G. Correlation of hormone receptor expression with histologic parameters in benign and malignant breast tumors. *Iran J Pathol.* 2015;10(1):23-34.
- [29] Mazouni C, Rimareix F, Mathieu MC, Uzan C, Bourgier C, André F, et al. Outcome in breast molecular subtypes according to nodal status and surgical procedures. *Am J Surg.* 2013;205(6):662-67.
- [30] Park S, Koo JS, Kim MS, Park HS, Lee JS, et al., Characteristics and outcomes according to molecular subtypes of breast cancer as classified by a panel of four biomarkers using immunohistochemistry, *The Breast.* 2011; 21:50-57.
- [31] Azizun-Nisa YB, Raza F, Kayani N. ER, PR & HER-2/neu (C-erb B 2) reactivity pattern with histologic grade, tumour size and lymph node status in breast cancer. *Asian Pac J Cancer Prev.* 2008;9(4):553-56.
- [32] Price JE. The biology of metastatic breast cancer. *Cancer.* 1990;66(6 Suppl):1313-20.
- [33] Song YJ, Shin SH, Cho JS, Park MH, Yoon JH, Jegal YJ. The role of lymphovascular invasion as a prognostic factor in patients with lymph node-positive operable invasive breast cancer. *Journal of Breast Cancer.* 2011;14(3):198-203.

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