

ASSOCIATION OF CLINICOPATHOLOGICAL PROGNOSTIC PARAMETERS WITH HORMONE RECEPTOR STATUS IN BREAST CARCINOMA

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ABSTRACT

Objective: To know the frequency of Estrogen, progesterone and HER-2neu receptor immuno expression in breast cancer, its molecular subtypes and association with the clinicopathological parameters.

Material and Methods: A total of 64 specimens of breast cancer were collected at Khyber Medical College in the study period from December 2015 to May 2016. To assess the morphological tumor characteristic, ER, PR & Her 2/neu status, Formalin fixed paraffin embedded sections were stained with Eosin Hemotoxylin and Monoclonal mouse antihuman anti-ER antibodies (code IR657/IS657), anti-PR antibodies (code IR068/IS068), and Her-2/neu by Hercep Test kit. ER, PR was assessed by Allred scoring system and HER-2 by Hercep method. Statistically frequencies and percentages of all the parameters were calculated with SPSS version 20. Association of Clinicopathological parameters with hormonal status was studied using Chi-square test.

Results: In total of 64 cases the mean age of the patient was 44.88 years \pm 12.22 SD. Among them 73% were premenopausal patients, including (40%) between 23-35 years. Histologically all were of invasive ductal carcinoma type. The estrogen, progesterone and HER-2 receptor positive cases was 87.5%, 76.6% and 13.4% respectively. Significant association of PR was found only with the premenopausal and postmenopausal status and with skin involvement with the p-values < 0.044 and < 0.040 respectively. However no significant correlation was seen with all other parameters. The correlation of ER, PR with HER-2 was also not significant. These cases were categorized as luminal A 79.7%, Luminal B 10.9%, HER-2 Positive 1.7% and triple negative 7.8%. There is inverse relationship between expression of ER, PR, and Her-2 neu.

Conclusion: Large number of patients was positive for ER and PR and may show good response to personalized therapy. Further larger analytical studies are required to evaluate the hormonal status of breast cancer and response to targeted therapy. HER-2/neu positive patients were few because of economic issues and lack of facilities, needs consideration on government level to help out the needy patients.

Key Words: Estrogen, progesterone, receptors, HER-2/neu (HER-2/neu).

This article may be cited as: Tasneem S, Ismail N, Khan SK, Khan MM. Association of clinicopathological prognostic parameters with hormone receptor status in breast carcinoma. *J Med Sci* 2016; 24: (3) 171-176.

INTRODUCTION

Breast cancer is the most common and frequently diagnosed disease in females globally accounting for 22% of all female cancers¹. It is also common in Pakistani females accounting for 30.9% according to

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Date Received: July 7, 2016

Date Revised: August 15, 2016

Date Accepted: September 1, 2016

tumor registry of Armed Forces Institute of Pathology (AFIP), Rawalpindi², constitute 33% of all cancers in females registered at the Nuclear Medicine, Oncology and Radiotherapy Institute (NORI) Islamabad³. Breast cancer is more common in fourth decade age group in Asian females as compared to western female population⁴. Studies have shown that at a younger age breast cancer has a more aggressive biological behavior and unfavorable prognosis as compared to older patients⁴. Progress is being made by utilizing biological markers for identifying targeted treatment. These biomarkers provide prognostic and therapeutic guidelines⁵. Measurable amounts of ER and PR are found in about 50-85% of patients with breast cancer. The frequency of positivity and the level of ER and PR increase with

age, reaching their highest levels in postmenopausal women⁶. Based on their expression four molecular types of breast cancer are recognized, Luminal A, Luminal B, HER2/neu positive (Non-Luminal), and triple negative breast cancer⁷. The prognosis and management of breast cancer depends on age, primary tumor size, histological type, grade and stage of the tumor, lymph node status, estrogen (ER), progesterone (PR) and HER-2/neu status⁸. These molecular types have significant correlation with prognosis and response to chemotherapy⁹. In this study we analyzed the frequency of Estrogen, progesterone and HER-2/neuimmunoexpression in breast cancer, its molecular subtypes and association with the clinicopathological parameters.

MATERIAL AND METHODS

A prospective descriptive study was carried out in department of Histopathology, Khyber Medical College from November 2015 to May 2016. Sixty four patients were inducted in the study, above 18 years of age. Patients who had metastatic/secondary carcinoma of breast or had neo- adjuvant treatment were excluded from the study.

Specimen were received in formalin and comprised of tru cut biopsies, partial mastectomy, modified radical mastectomy and formalin fixed paraffin embedded blocks (FFPE) along with Histopathology report of breast cancer. Gross examination of specimen was carried recording status of skin, primary tumor size in cm and number of axillary lymph nodes recovered. Representative sections from tumor and lymph nodes was taken and submitted for further processing. 3-5µm thick sections were stained with H&E for histological evaluation including histological typing of tumor, grading of tumor according to modified Bloom and Richardson scoring system¹⁰. Lymph nodes were evaluated histologically for metastasis. TNM staging was provided with the clinical data. Immunohistochemistry was done for ER, PR & HER-2/neu using Monoclonal mouse anti-human anti- ER antibodies (code IR657/IS657), anti-PR antibodies (code IR068/IS068), and Her-2 by Hercep Test kit by taking 3-4µm thick sections from FFPE.

ER, PR was assessed by Allred scoring system by taking into account sum of percentage of cells stained and intensity of staining. ER and PR were considered positive when >1% cells stained positive, total score of ≥3 is considered positive¹¹. Hercep Score 3+ was taken as Positive, 2+ as equivocal, and 0 or 1+ as Negative for HER-2/neu.

SPSS 20 was used for data entry and statistical analysis. Pearsons Chi-square test was applied to assess the correlation of the clinicopathological variables like age, tumor size, grade, depth of invasion, skin

involvement, lymph node metastasis and stage of the disease with ER, PR and HER-2/neuimmunoexpression. Differences were considered as statistically significant at P values of <0.05 as in other studies.

RESULTS

A total of 64 cases of breast cancer were evaluated for Estrogen, progesterone and HER-2/neureceptor status. Frequencies of Hormone in breast cancer is shown in Table 1. The cases were collected from Pathology Department, Khyber Medical College, where FFPE blocks, biopsy, mastectomy and MRM specimens were received from December 2015 to June 2016 for immuno histochemistry. The mean age of the patient was 44.88 with SD ±12.22. The premenopausal patients were 47 (73%) and postmenopausal were 17 (27%). In premenopausal group 19/47(40%) were between 23-35 years. Maximum number of patient were of tumor size Correlation of clinicopathological parameters with ER, PR and HER-2/ neu status is shown in Table 2. 2-5cm (55%) followed by (T3) 27%. Most of the tumor were of grade II (57.8%) followed by grade III (42.8%). Large number of patients were diagnosed in stage II disease (51%) followed by stage III (25%). Skin was involved in 8(12.5%) cases. Lymph nodes were not assessed in 17 cases as we received mostly blocks or biopsy specimens. Where ever reports were available with the cases we recorded the lymph node status and stage of the disease. In 48 cases lymph nodes were retrieved, among these 17 cases had no metastatic disease. Maximum number of lymph nodes involved by metastatic disease was 1-3 (48%).

The estrogen receptor positive cases were 56(87.5%) and the rest were negative. PR positive cases were 49(76.6%) and HER-2/neu positive were 8/60 (13.4%). When these are correlated with the prognostic parameters Relationship of HER-2/neu with ER & PR is shown in Table 3 significant P-values of 0.044 were seen in only age group i.e premenopausal and postmenopasal status with PR and also with the skin involvement. The correlation of ER, PR with HER-2/neu was also not significant. On the basis of hormonal status, these cases were categorized as Luminal A 51 (79.7%), Luminal B (10.9%), HER-2/neu Positive 1 and triple negative 5 (7.8%) Table 4.

Table 1: Frequencies of Hormone in breast cancer

	No (%) Positive	No (%) Negative	Total
ER	56 (87.5%)	8 (12.5)	64
PR	49 (76.6)	15 (23.4%)	64
HER-2	8 (13.4)	52 (86.6%)	60

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Table 2: Correlation of clinicopathological parameters with ER, PR and HER-2/ neu status

Parameters	Status of ER value		Total N (%)	Status of PR value		Total	Status of HER-2/neu value		Total
	Positive N (%)	Negative N (%)		Positive N (%)	Negative N (%)		Positive N (%)	Negative N (%)	
Age : 23-35 years	17(89)	2(11)	19(100)	17(89)	2(11)		4(22)	14(88)	18(100)
Meno-pause Status									
Premeno-pausal 23-50 years	41(87.2)	6(12.8)	47	39(83)	8(17)	47	7	37	44
Post-meno-pausal	15(88.2)	2(11.8)	17	10(59)	7(11)	17	1	15	16
P Value	0.915			0.044			0.330		
Tumor size									
1-2 cm	11	1	12	11	1	12	0	12	12
2.1-5 cm	31	4	35	25	10	35	7	26	33
5.1-11 cm	14	3	17	13	4	17	1	14	15
P Value	0.727			0.361			0.123		
Grade									
I	7	0	7	7	0	7	1	5	5
II	34	3	37	28	9	37	4	31	35
III	15	5	20	14	6	20	3	16	19
Total									
P Value	0.105			0.267			0.875		
Stage									
I	13	1	14	10	4	14	0	14	14
II	29	4	33	26	7	33	6	24	24
III	13	3	16	12	4	16	2	13	15
IV	1	0	1	1	0	1	0	1	1
P-value	0.780			0.891			0.326		
Skin involvement									
Positive	6	2	8	4	4	8	2	6	8
Negative	47	9	56				7	45	52
P-value	0.531			0.040			0.503		
Lymph Node									
No positive lymph nodes	16	1	17	6	17		0	17	17
1-3	22	1	23	20	3	23	3	17	20
4-9	5	2	7	6	1	7	2	5	7
>9	1	0	1	1	0	1	0	1	1
NX	13	4	17	12	5	17	3	6	9
P Value	0.132			0.336			0.214		

Table 3: Relationship of HER-2/neu with ER & PR

HER-2	ER		Total	HER-2/neu	PR		Total
	Positive	Negative			Positive	Negative	
Positive	2	6	8		1	14	15
Negative	6	46	52		7	38	45
P value	0.288				0.350		60

Table 4: Frequency of molecular subtypes of breast carcinoma

Luminal A	(ER+/PR+/HER-2-	51 (79.7%)
Luminal B	(ER+/PR+/HER-2+)	7 (10.9%)
HER -2 +	HER -2 +	1 (1.7%)
Triple Negative	(ER-/PR-/HER-2-)	5 (7.8%)

DISCUSSION

The present study was designed to determine the association of molecular subtypes of breast cancer with clinicopathological prognostic markers such as age of the patient, primary tumor size, histologic grade, axillary lymph node status, disease stage, pattern of distribution of ER, PR and HER-2/neu in breast cancer. Histologically all the cases were invasive ductal carcinoma which is the commonest type 13.

In the present study, mean age of the patient was 44.88 which is similar to other national studies conducted by Faheem et al¹⁴ and Akbar et al¹⁵. Youngest patient was 23 years old. Large number of patients 73% were premenopausal and 27% were postmenopausal. 40% of patients from premenopausal group were ≤35 years. This is in contrast to the statistical report 2003-2007 of Surveillance Epidemiology and End Results (SEER) from national cancer institute of United States, reported that the mean age was 61 years with only 1.9% of total breast cancer patients were below 34 years and 32% between 35-54 years of age¹⁶. Trend towards younger age group as compared to western studies may be due to genetic variability, aggressive tumor biology and hereditary factors which needs to be explored by conducting larger nationwide studies.

Frequency of ER positivity was 87.5%, i.e in large group of patients relative to the other studies. In the study by Kamil et al (32%)¹ Sharif et al¹⁶. Showing 74% positivity and Chung et al (61%)¹⁷. Expression of PR was positive in 76.6%, higher in our patients relative to other studies by Kamil et al (29.68%)¹ Sharif et al 67%¹⁶ and Chung et al (51.9%)¹⁷. Her2/neu positivity was seen in 13.4% of patients which is identical to European studies (13%)¹⁸ but lower as compared to studies conducted by Pinjwani et al¹⁹, Mostafa et al²⁰ and Ross et al²¹. Her-2/neu positivity was quite low in our study, as we considered 2+ as negative because of economic issues and lack of facility for FISH test. This difference in hormone expression may be due to smaller sample size which could be addressed by conducting larger studies. It could also be due to racial, regional and social differences.

In the present study association of age with hormone expression was sought. The patients in premenopausal and postmenopausal group showed significant correlation ($p < 0.044$) with PR expression. Greater number of patients was positive for PR in the premenopausal group. Correlation was not significant with ER and Her-2/neu. This is in contrast to study by Faheem et al where patients in premenopausal group showed significant correlation ($p < 0.5$) to ER and PR expression. The reason for the difference could be the same as mentioned earlier. Her2/neu positivity was 61.6% in premenopausal and 25% in postmenopausal group. These results were similar to study results of Faheem et al and Hussain et al²² stating high expression of Her-2/neu in premenopausal women.

Frequency of molecular subtypes was as follows in our study; Luminal A 79.7%, Luminal B 10.9%, Non Luminal 1.6%, Triple Negative breast cancer 7.8%. Luminal A is the most frequent molecular subtype of breast cancer in our study which is similar to international studies²³. Frequency of Triple negative subtype in present study was 7.8% which is nearly similar to study by Shaukat Khanum Hospital (11%)²⁴ for the reason that equivocal cases were not tested for FISH as the facility was not available locally and secondly it was economically not possible for patients.

No significant correlation was noticed with tumor size and lymph node status. Skin was positive for tumor in 12.5% of cases and showed significant correlation with PR receptor expression ($p < 0.040$). Majority of patients i.e. 57.8%, had Grade II breast cancer, followed by Grade III. The correlation of tumor grade with ER PR and Her-2/neu was insignificant.

Disease stage was also studied with majority of patients presenting in Stage II (51.56%), in Stage III (25%) followed by Stage I (21.9%) and Stage IV (1.6%). This was similar to studies conducted in Shaukat Khanum Hospital with 59% of patients presenting in Stage II and 33% in stage III disease²⁴. This is in contrast to patients presenting in United States 60% of the patients present in early stage whereas only 5% present in late stage²⁵. Correlation of disease stage with ER, PR and Her-2/neu was found insignificant. Most of the patients presented late due to lack of awareness, poverty and lack of health facilities.

CONCLUSION

Breast cancer is a major health problem in Pakistani females. Patients are presenting late due to lack of medical facilities, socioeconomic constraints and lack of public awareness. The expression pattern of hormone receptors i.e ER, PR in southern region of Khyber Pakhtunkhwa is relatively high and HER-2 neu

is low. Immunohistochemical studies are not available in Public sector tertiary care hospitals.

RECOMMENDATION

Public awareness programmers, health education and breast cancer screening clinics should be setup in all the cities across the country. Larger studies in Khyber Pakhtunkhwa should be under taken to study tumor biology, clinical course with follow up so that better diagnostic and therapeutic modalities are set up in public sector tertiary care hospital in the best interest of public.

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CONFLICT OF INTEREST: Authors declare no conflict of interest

GRANT SUPPORT AND FINANCIAL DISCLOSURE NIL

AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Tasneem S: Main idea, concept and Study design.

Ismail N: Data collection and follow up.

Khan SK: Statistics and bibliography.

Khan M: Operating Surgeon.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

The Journal of Medical Sciences, Peshawar is indexed with WHO IMEMR (World Health Organisation Index Medicus for Eastern Mediterranean Region) and can be accessed at the following URL.

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