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Original Article

Immunohistochemical Study on Hormone Receptors and HER2 Status in Invasive Breast Carcinoma and its Therapeutic Implications

Md Iqbal Karim[1*], Md Tarikul Islam[2], Nazlima Nargis[3]

Abstract:

Background: *Immunohistochemistry (IHC) has become an integral part of histopathology in the diagnosis of biopsy sample. Although haematoxylin and eosin (H & E) stain remains the fundamental basis for diagnostic pathology of the breast, IHC stains provide useful and sometimes vital information. Moreover, the role of hormonal therapy in hormone receptor–positive breast tumours, as well as targeted chemotherapeutic agents for human epidermal growth factor receptor-2 (HER2) positive cases, IHC studies represent a major part of workups.*

Objective: *The study was carried out to identify the common immunohistochemical markers in invasive breast carcinoma and to find out the relationship between hormonal receptor status and HER2 over expression with the grade of tumour and its therapeutic implications.*

Method: *In the present study, immunohistochemical assay of total seventy-two blocks of breast carcinoma patients was performed to know the hormone receptors and HER2 status as well as histological examination.*

Result: *72 samples were grouped to study hormonal status and their relation with clinicopathological factors. Outcome of this study documented 58.33 %, 44.44% and 25 % expression rate of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2) respectively. The negative expression of HER2 receptors found higher (75%) than ER & PR. The ER, PR negative and HER2 Positive cases found in high grade breast cancer (Grade-3). An inverse correlation of HER2 expression with ER and PR expression was observed (p=0.001).*

Conclusion: *The role of hormone receptors and HER2 repression as a prognostic and therapeutic tool in breast cancer is widely accepted and effective for patients.*

Keywords: *Breast carcinoma, Estrogen, Progesterone, HER2, Immunohistochemistry.*

Introduction:

Breast carcinoma is a major concern and one of the leading causes of cancer related death worldwide. Breast carcinoma like many other types of cancer is a complex heterogeneous disease controlled by a multitude of genetic and epigenetic alterations[1].

During last few decades, the mortality rate has declined significantly, primarily due to the early use of adjuvant chemotherapy as well as detection of earlier stage tumours due to increased screening[2]. Prognosis and management of breast carcinoma are influenced by the classical variables such as histological type and grade, tumour size, lymph node status and status of hormonal receptors like Estrogen Receptor (ER) and Progesterone Receptor (PR) of the tumour and more recently human epidermal growth factor receptor-2 (HER2) oncoprotein status[3]. The ER, PR and proto-oncogene HER2 profile of a female primary breast carcinoma play a significant role as a predictive marker in patient management. The hormone receptors and HER2 status at the time of initial diagnosis have been established as a clinically useful, standard-of-care parameter in determining treatment options and subsequent patient response. Current therapeutic strategies for management of primary breast carcinomas rely on the accurate Immuno-Histo-Chemical (IHC) determination of hormone receptor status in order to determine the clinical utility of hormone-directed therapies such as Selective Estrogen Receptor Modulators (SERMs) that improves the disease free survival in women with hormone receptor-positive tumors[4]. In addition to hormone receptors, HER2 has emerged in recent years as an important independent predictive marker in primary breast carcinoma.

The interrelationship of ER, PR status, and HER2 overexpression has an important role in the management of breast carcinoma. ER/PR status is inversely related to HER2 status. Survival and response to hormone therapy (tamoxifen) are more favorable among women who are receptor positive, intermediate for tumours discordant on receptor status and less favorable for receptor negative patients. However, if there is an amplification of both ER and HER2, then the patient would not respond to tamoxifen[5]. Tamoxifen acts as an

1. Classified Specialist in Pathology, Department of Histopathology, Jaber Al Ahmed Armed Forces Hospital, Kuwait. Deputation to Medical, OKP-5, Kuwait.
2. Graded Specialist in Surgery, OKP-5, Kuwait.
3. Associate Professor, Department of Gynae & Obstetrics, Ibn Sina Medical College, Dhaka.

***Corresponding Author:**

Lt Col (Dr) Md Iqbal Karim
Classified Specialist in Pathology,
Medical OKP-5, BMC to Kuwait
Phone : +96566325942,+88 01718417441
Email: iqbalhistopath@gmail.com

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agonist if there are both ER expression and HER2 expression and cause proliferation of the tumor tissue, leading on to the resistance of tamoxifen[6]. In such cases, it has been shown that trastuzumab therapy is more effective. Patients with lone HER2 overexpression are also candidates for trastuzumab[7]. Thus, IHC studies have a role as a decision maker in the targeted therapy.

The aim of study was to identify the common immunohistochemical markers in invasive breast carcinoma and to find out the relationship between hormonal receptor status and HER2 overexpression with the grade of tumour and its therapeutic implications. The grading system that's usually used is as follows: grade I – cancer cells that resemble normal cells and aren't growing rapidly. grade II – cancer cells that don't look like normal cells and are growing faster than normal cells. grade III – cancer cells that look abnormal and may grow or spread more aggressively.

Materials and Methods:

A retrospective study was conducted from January 2017 to December 2018 in the department of Pathology, Jaber Al Ahmed Armed Forces Hospital and Adan Hospital, Kuwait. Total 72 cases were added in this study. Clinical details were archived from the files. Specimens were routinely fixed 24-48 hours in 10% neutral buffer formalin. They were examined grossly and representative tissue bits were taken according to standard guidelines and then processed. Sections were stained with routine H&E stain. IHC analysis of hormone receptor assay and Her2 status was done on the paraffin-embedded tissue blocks by the supersensitive polymer HRP system based on non-biotin polymeric technology. The present study was conducted after obtaining the ethical approval from the Ethical Review Committee.

IHC stained slides were evaluated for the presence of reaction, cellular localization (nuclear or cytoplasm), pattern of staining (focal or diffuse) and intensity of reaction in the individual tumor cells (strong or weak). Scoring for ERs and PRs was done using Quick score system. Quick score system uses two principles, intensity and proportion. The quick score system based on intensity is as follows: When there are no staining - score 0. Weak stain- score 1, moderate stain - score 2, and strong stain - score 3. The staining system based on the proportion of stain is as follows: 1% nuclear stain - score 0, 1-10% stain-score 2, 11-33% - score 3, 34-66% - score 4, and 67-100% - score 5. This comes with a maximum score of eight. Score of more than two is considered as positive. The advantage of this score is that it correlates with the probability of response to endocrine therapy. For HER2 scoring the following rule was followed: No staining or incomplete membrane staining and faint/barely perceptible in ≤10% of the tumor cells – HER2 negative. Incomplete and faint membrane staining in >10% of the invasive tumor cells are taken as HER2 1+. A weak to moderate complete membrane staining in >10% tumor cells are graded as HER2 2+. A strong complete membrane staining in> 10% tumor cells are graded as HER2 3+.[8]

The statistical analysis was performed with Statistical Package for Social Science (SPSS) software version 11. The Pearson Chi-square test was used to compare the possible correlation between ERs, PRs, and HER2 with tumor size, nodal status and histological grades. P value ≤ 0.05 was considered as significant.

Results:

Total 72 cases of breast cancer were analyzed in this present study. The age ranges between 20-80 years, divided the age into 6 groups showing in Table 1 & Distribution of breast cancer according to age are shown in Table 2.

Table 1 :

Age (yrs)	Number of cases	% of cases
20-30	6	8.33%
31-40	10	13.89%
41-50	18	25%
51-60	14	19.45%
61-70	16	22.22%
71- 80	8	11.11%
Total	72	100%

Table II :

Age	Invasive Ductal Carcinoma	Invasive Lobular Carcinoma	Mucinous carcinoma	Medullary carcinoma	Inflammatory carcinoma	Tubular carcinoma	Metaplastic carcinoma	Total
20-30	5	1						6
31-40	6	2	1	1				10
41-50	11	2	2	1	1	1	-	18
51-60	8	1	2		1	1	1	14
61-70	9	2	1	1	1		2	16
71+	4	1	1		1		1	8
Total	43	9	7	3	4	2	4	72

Immunohistochemical analysis (ER, PR and HER2 study) of all breast cancer specimen was performed and their relation to clinicopathological factors were studied in detail. HER2 expression was categorized according to their intensity of membrane staining into 1+(negative), 2+(equivocal), 3+ (positive) shown in table-III. Equivocal Samples were sent for FISH analysis and were confirmed as positive. Total 18 cases (25%) were HER2 positive and 48 cases were true negative.

Table III :

HER2 status	Staining pattern 1+ (Negative)	2+ (equivocal)	3+ (Positive)	Negative
	06	08	10	48

In Table IV the expression rate of ER, PR and HER2 receptors are shown. HER2 negative expression was found higher than positive expression (75%). An inverse correlation of HER2 expression with ER and PR expression was observed (p=0.001).

Table IV:

Receptors	ER	PR	HER2	p-value
Positive	42 (58.33%)	32 (44.44%)	18 (25%)	0.001*
Negative	30(41.67%)	40(55.56%)	54(75%)	

Correlation of ER, PR and HER2 oncoprotein overexpression with grade of tumour are shown in Table V.

Table V :

Group	No of cases (N=72)	HER2 Positive (N=18)	HER2 Negative (N=54)	Tumour grade
ER+/PR+	28	01	27	2
ER+/PR-	20	02	18	2
ER-/PR+	04	01	03	3
ER-/PR-	20	14	06`	3

Discussion:

Breast cancer is the most common cancer with increased mortality rate. In addition to pathological grade and stage, breast cancers are routinely assessed for hormone receptor status (ER) by immunohistochemistry and human epidermal growth factor receptor 2 (HER2) expression by IHC or amplification by FISH in order to guide the choice of the appropriate adjuvant therapy[9]. This is routinely done in every case of invasive breast carcinoma because patients with ER positive primary breast tumours are offered adjuvant hormone therapy, routinely tamoxifen for five years, while post menopausal women may receive aromatase inhibitor. Patients with overexpression of Her2 Neu are eligible for trastuzumab that targets the Her2/Neu receptor[10,11].

Breast carcinoma is the most common carcinoma in women and accounts for 22% of all female cancers which is more than twice the prevalence of cancer in women at any other site. In our study most of the patients were below 50 years of age which is quite similar to many local and international studies[12,13].

Approximately 75% of all Breast Cancers (BC) express ER and/or PR[14] while up to 20% of breast cancer show an over expression/amplification of HER2[15,16]. Almost similar findings were found in our study where Her2/neu2 expression was 25%.

ER is expressed in 70–80% of invasive ductal carcinoma, while PR is expressed in 60–70% of invasive breast carcinoma[17]. In our study the ER was expressed in 58.33% of cases. While PR was expressed in 44.44% of cases and Her2/Neu was expressed in 25% of cases. The expression of ER and PR is quite comparable to many international and local studies. The study conducted by Fatima et al.[18] also showed ER positivity to be 52.4% very close to our study. Similarly a study conducted in India by Desai et al[19] showed ER positivity in 33% of cases, 46% were PR positive. In their study ER and PR immune reactivity increased with advancing age. Ranatunga et al[20] observed ER and PR positivity in 53% and 50% of cases respectively. Ong et al[12] reported 60% of the cases were ER positive.

In our study most of the patients were between 25–45 years and in premenopausal age group where ER positivity is lower and it increases in post– menopausal age group and carcinomas were of high grade.

The frequency of HER2 reactivity is 25% which is very much higher which is similar to study conducted by Geethamala,[21] it was 25%.

In our study triple negative cases were 8.33% and all were of grade 3, while study conducted by Suvarchalaet al.[13] its quite higher, 42.1% as compared to other studies. They attributed this to differences in techniques of evaluation, higher tumour grades and majority being menopausal women.

Considering the grade of tumour and hormonal receptors, mostly patients of grade 2 and hormonal receptors was positive which is inconcordance with study by Baswal et al [21] In our study grade 3 tumours were either triple negative or Her2/Neu positive which is again inconcordance with other studies like Geethamala et al [21].

Since breast carcinoma is a steroid hormone–dependent tumor it is obvious that sex steroid hormones, especially estrogen, play an important role in the development of breast cancer. Hormone receptor status is one of the most important prognostic factors affecting the quality of life (QOL) for breast cancer patients. An increase in hormone receptor expression is associated with inhibition of cell proliferation and subsequently weakens tumorigenesis. ER^+ tumors show a lower incidence of recurrence and a longer disease-free interval, regardless of tumor size or lymph node status[23]. However, the prognostic value of PR status is controversial. It is important to take the PR status be taken into account with ER status because patients with both ER^+ and PR^+ tumors usually have a better prognosis than patients with ER^+ and PR^- tumours[24].

Approximately half of BC that overexpresses HER2 also

expresses Hormone Receptors (HRs). Although HR positivity predicts efficacy of endocrine agents, preclinical and clinical data suggest that HER2 overexpression confers intrinsic resistance to hormonal treatment. In addition, HER2 overexpression is an independent adverse prognostic factor regardless of the hormonal status of the tumour, indicating that patients with $HR^+/HER2^+$ breast tumours might not derive a benefit from single-agent hormonal therapy. These data provided a strong rationale for exploring the targeting of both HR and HER2 signaling pathways in $HR^+/HER2^+$ breast tumours to optimize hormonal therapy and overcome resistance to anti-estrogen therapy. Results from a randomized clinical trial that combined hormonal treatment with targeted anti-HER2 therapy in postmenopausal women with $HR^+/HER2^+$ advanced BC indicate that this novel dual-targeting strategy significantly improves outcomes compared with endocrine treatment alone. Nonetheless, other data suggest that it might achieve an inferior outcome compared with anti-HER2 therapy plus chemotherapy. Therefore, targeting both the HR and HER2 signaling pathways up front might not be the most-effective therapeutic strategy in the management of $HR^+/HER2^+$ BC. We discuss the clinical implication of resistance to endocrine therapy, and describe new insights into the management of $HR^+/HER2^+$ advanced BC[25].

Conclusion:

The role of hormone receptors and HER2 repression as a prognostic and therapeutic tool in BC is widely accepted and effective for patients. Our study confirms that receptor expression of ER, PR and HER2 is significantly associated with tumor grade. An inverse correlation of HER2 expression with ER and PR expression was also observed. Functional analyzes of ER, PR and HER2 receptors are needed to apply appropriate hormonal treatment that significantly improves the quality of patient's life.

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