RESEARCH ARTICLE

AN ANALYSIS AND SIGNIFICANCE OF IMMUNOHISTOCHEMISTRY ER, PR AND HER2 TESTING FOR BREAST MALIGNANCY IN A TERTIARY CARE CENTER

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Introduction:

Breast malignancy is the most common malignant tumor and the second most common cause of carcinoma death in women, with more than 1.7 million cases occurring worldwide annually.[1] The large majority of breast cancers are detected during the post menopausal years. However, breast cancer can develop at any age from childhood to old age. [1] Hormone receptor studies such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2(HER-2/neu) are routinely done in breast carcinoma.[2] A correlation between the presence of hormone receptors in the tumor and response to hormonal therapy and chemotherapy is a significant development in the breast carcinoma management.[3] In our study analysis of receptor studies and expression pattern pertaining to the age group in our zone.
Materials And Methods:-
In our study we included all cases of breast malignancy (296 cases) for one year period (from January 2019-January 2020) received from surgical department. Clinical parameters of patients such as age, sex and parity were taken. All the modified radical mastectomy specimens and tru cut biopsy specimens were submitted for histopathological processing. All the tissues were fixed in 10% buffered formalin immediately, after sections were taken from tumor and biopsy were submitted for processing. Routine haematoxylin and eosin staining was performed for histopathological diagnosis. Histopathological type of the tumor were classified according to WHO.

Immunohistochemistry (IHC) for ER ,PR and HER2/neu were performed in the respective block of paraffin embedded tumor tissue. 4 micrometer thick sections were taken on poly-L-lysine coated slides and submitted for IHC. After antigen retrieval was done using citrate buffer at pH2.5 for hormone receptor and pH 6 for HER2/neu. They were then incubated for 30 minutes with primary monoclonal antibodies against HER2/new (DAKO, clone 124,1:100), ER (DAKO, clone 1D5,1/25) and PR (DAKO, clone PgR636, 1/50) followed by incubation with biotin – secondary antibodies. The streptavidine –peroxidase complex was visualized using di amino benzidine as a chromogenic substrate. The normal breast ducts served as a internal positive control for ER/PR. Breast malignancy with known HER2 /neu over expression served as an external positive control for HER2/neu staining. For interpretation of ER/PR staining the following Allred system of scoring method was used as in Cap protocol.

| Table 1:| Allred score for Estrogen and Progesterone Receptor evaluation: |
|-------|-------------------------------------------------|
| 0,2   | Negative                                        |
| 3, 4, 5, 6, 7, 8 | Positive                                     |

| Table 2:| For interpretation of HER2/neu staining the following methods were used. |
|-------|--------------------------------------------------------------------------------|
| The H score is determined by multiplying the percentage of cells demonstrating each intensity (scored from 0 to 3) and adding the results. There are 300 possible values. In this system, <1% positive cells is considered to be a negative result. |

| Calculation of H Score |
|------------------------|
| Percentage of cells | Values multiplied |
| Cells with no signal  | 0      | %x0=0 |
| Cells with weak signal| 1      | %x1=1 |
| Cells with moderate signal | 2      | %x2=2 |
| Cells with strong signal | 3      | %x3=3 |

Results:-
This study group comprised of a total 296 breast malignancy. Mostly occurred in females (294/296) for about 99% and male (2/296) for about 1%.Out of 296 cases 95 cases were between 40-50 years of age followed by 84 cases from 51-60 years of age group.

| Table 3:| Age wise distribution of cases. |
|-------|---------------------------------|
| Age( in years) | Total cases | Percentage |
| 20-30             | 8               | 2.7%       |
| 31-40             | 53              | 18%        |
| 41-50             | 95              | 32%        |
| 51-60             | 84              | 28.3%      |
| 61-70             | 41              | 13.8%      |
| 71-80             | 14              | 4.7%       |
| 81-90             | 1               | 0.1%       |

The most common histological type was invasive carcinoma breast no special type 290/296(97.9%). Next stands the invasive papillary carcinoma 4/296(1.3%), the carcinoma with medullary feature 1 case and mucinous carcinoma,1case.

According to the molecular classification of breast malignancy, the IHC expressing profile pattern was analyzed,[Table 4], [Figure 3]
Table 4: Results of the Molecular classification of ER/PR and HER2/neu markers.

| Markers          | Number of cases | Percentage |
|------------------|-----------------|------------|
| ER +ve HER2 –ve  | 73              | 24.6%      |
| ER+ve HER2 +ve   | 68              | 22.9%      |
| ER-ve HER2+      | 64              | 21.6%      |
| Triple negative  | 71              | 23.9%      |
| Triple positive  | 20              | 6.75%      |
| Total            | 296             |            |

From the above table we infer that the ER+ve HER2 –ve tumor were 73/296(24.6%) [Figure.1]

Figure 1: ER Expression-Invasive carcinoma breast, A nest of tumor cells showing intense nuclear staining, (IHC Stain), X 40.

ER+ve HER2 –ve were 68/296 cases. ER-ve HER2 +ve were 64/296 cases(21.6%)[Figure.2]
Figure 2: Invasive carcinoma breast. The highly atypical hyperplastic cells overexpress with HER 2/neu showing the intense and circumferential cell membrane staining, (IHC Stain), X 40.

Triple negative tumor cases were 71/296 (23.9%) and triple positive cases were 20/296 (6.75%).

The pure ER positivity in 141/296 cases (47.6%), PR positivity in 69/296 cases (23.3%) whereas HER2 neu positivity cases 132/296 cases (44.5%). In our present study, both ER and PR positivity was found in 66 cases (22.2%) and both negative status in 70 cases (23.6%).

We analysed age wise distribution of expression pattern [Table 5]. This was analysed between the age group of 20-90 years. In this, the highest number of cases was found in the age group of 41-50 years, that is about 95 cases were reported. Here in this range ER+ve HER2neu +ve was 35.7%. 84 cases were reported in the age group of 51-60 years where ER+ve and HER2 -ve was 27 (32.1%). In the age group ranging from 31-40 years 53 cases were reported and it was found that it is expressed in all ER/PR and HER2 markers. The least number of case (1 case) was found in the age group of 81-90 years which was triple negative. This same characteristics were found in the age group of 20-30 years. Thus from the above analysis it is concluded that triple negative was found only in extreme age groups (20-30 years and 80-90 years).

Table 5: Age wise distribution of expression pattern According to the molecular classification.

| Age      | ER+ve HER2 –ve | ER+ve HER2+VE | ER-ve HER2+ve | Triple negative |
|----------|----------------|---------------|---------------|-----------------|
|          | Total cases    | %             | Total cases   | %               | Total cases    | %               |
| 20-30    | 0 cases        | 0%            | 2             | 25%             | 4              | 50%            |
| 31-40    | 14 cases       | 26.4%         | 13            | 24.5%           | 13             | 24.5%          |
| 41-50    | 17 cases       | 17.8%         | 34            | 35.7%           | 21             | 22.1%          |
| 51-60    | 27 cases       | 32.1%         | 8             | 21.6%           | 21             | 25.3%          |
| 61-70    | 13 cases       | 31.7%         | 10            | 24.3%           | 8              | 19.5%          |
| 71-80    | 6 cases        | 42.8%         | 4             | 28.5%           | 1              | 7%             |
| 81-90    | 1 case         | -             | -             | -               | -              | 1              |

Discussion:-

Invasive ductal carcinoma is the largest group of malignant mammary tumors, comprising approximately 75% of mammary carcinomas. In the later WHO lexicon, the term invasive ductal carcinoma is replaced by invasive carcinoma of no special type (NST), with authors collective opinion “The use of the term DUCTAL perpetuate the traditional but incorrect concept that these tumors are derived exclusively from mammary ductal epithelium in
distinction from lobular carcinomas, which were deemed to have arisen within the lobules, for which there is also no evidence” [4].

As a general rule, immunohistochemistry cannot and should not replace the histopathology. It is however a valuable adjunct in diagnosis of breast pathology. The American society of clinical oncology has issued consensus panel statements supporting the use of a three-tiered categorization of ER staining percentages that acknowledges the existence of both “positive” and “low positive” cases. Additionally, the National Institute of Health consensus statement on Adjuvant Therapy for Breast cancer also states that “any degree of ER nuclear staining detected by immunohistochemistry should be considered a positive result” [5-6].

Using IHC with monoclonal antibody against HER-2/neu receptor, a positive result is interpreted as 3+ which is characterized by strong, complete cell membrane (“chicken wire”) staining. While an immune score of 2+ is defined as weak to moderate, mostly incomplete cell membrane reactivity, a score of 1+ represents a weak cytoplasmic and are incomplete cell membrane reaction. Using IHC, at least 10% tumor cells should reveal a score of 3+ in order to be reported as HER2/neu over expression [7].

Global gene expression profiling of breast cancer has demonstrated that invasive carcinoma breast no special type tumor can be classified into subtypes on the basis of expression pattern. At least 3 main molecular classes have been identified: Luminal/ER+ (60-70%), HER2+ (15-20%), Basal-like/triple negative (15-20%) [8]. But in our study ER+ luminal type was 47.5%. HER2 +ve tumor was 22.9% and triple negative tumor was 23.9%. While comparing our study with the statement given by David J.Dabbs, the molecular pattern differed by showing an increase in the incidence of HER2+ and triple negative tumor.

A prevalence of 32.6% for ER+ve and 46.1% for PR+ve breast cancer has been documented in a study carried out in India [9]. In our study the PR positivity rate was 34.12% which was less when compared with the reference given [9]. Many study reports showed that the expression of hormone receptors increased in age group of the patient [10-15]. In our study the ER expression is increased in higher age group and is better correlated with our study.

As the age increased, the percentage of women with ER+ve/HER2 –ve subtype also increased [16]. It is well correlated with our study. In comparison with the ER+ve, HER2 +ve and triple negative subtypes as age increased the percentage of cases decreased [16]. This is a contrast feature in our study. In both extremities of ages Triple negative tumor was in higher percentage.

**Significance of luminal cancer:**
Luminal tumors are reminiscent of “Normal luminal epithelial cells” including CK8/18+. Lum A are ER+ve and are enriched with genes associated with active ER pathway, low levels of proliferation related genes, low histological grade and generally good prognosis. The Lum B tumors are high grade with high proliferative index and worst outcome [17].

**Significance of HER2+ve tumor:**
The most promising and useful findings are based on recent studied showing HER2+ve cancers respond favorably to new antibodies based therapies, targeting specifically the HER2 protein, such a Transtuzumab [18-19].

**Significance of basal-like cancer:**
The basal-like subtype more commonly occurs in younger individuals [20]. Our age group also follows such pattern. The tumor usually shows high response to cytotoxic chemotherapy, although the majority relapses and the overall prognosis is very poor [20].

**Conclusion:**-
Breast malignancy is the most common malignant tumor and the second most common cause of carcinoma death in women. In conclusion, the marker status of ER/PR and HER2/neu is gold standard for all breast malignancy for the purpose of diagnosis, prognosis and therapy.

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Competing Interests
None Declared.

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