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Study of Androgen Receptor as a Quadruple IHC Marker in Breast Carcinoma in a Tertiary Care Centre

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ABSTRACT

Breast tumor is second most common tumor in the world. Breast tumor may be defined on the ground of absence or presence of Estrogen receptor (ER), Progesterone receptor (PR), and over expression of Human epidermal growth factor receptor 2 (HER2). Absence of these hormonal receptor (ER, PR, and HER2), define as TNBC (Triple negative breast carcinoma). TNBC can be classified in four subtypes, Basal 1, Basal 2, Mesenchymal and Luminal androgen receptor (LAR). LAR is most perceptive for AR antagonists because of it depends on AR signaling pathway. AR perform major role in breast carcinoma treatment, AR Antagonist predominantly used in Tamoxifen (SERM) resistant and TNBC. BR grade and HR status of 80 surgically resected specimen of carcinoma breast were determined by routine histopathological examination and IHC. Allred scoring system were employed to evaluate HR status. Chi square test was applied to find association between dependent and independent variables and $p<0.05$ considered as level of significance. In all cases of breast carcinoma, androgen receptor was positive for 89.7%, 82.4% and 52.6% of ER, PR and Her 2 positive cases. Significant association was found between AR status and ER/PR status ($p<0.05$). The present study show androgen receptor (AR) is currently emerging as a new biomarker and a potential new therapeutic target in the treatment of breast carcinoma, and the findings of this study could have prognostic and therapeutic implications for treatment protocols that include androgen receptor agonists or antagonists in appropriate clinical settings.

INTRODUCTION

Breast cancer is more common non-skin malignancy in women, and it is the second leading cause of cancer death after lung cancer. Breast cancer has a one-in-eight probability of developing in a woman who lives to be 90 years old[1]. In 2020, 2.3 million women worldwide were diagnosed with breast cancer, with roughly 7 lakh mortalities[2].

Breast cancer is the most common cancer among Indian women, with a prevalence of 25.8 per 100,000 women and a fatality rate of 12.7 per 100,000 women. Breast cancer was responsible for nearly one out of every four cancer fatality in Indian women. Breast cancer is most prevalent in females aged 50 to 69 (46.5 %)[3].

Age, obesity, hazardous alcohol use, family history of breast cancer, history of exposure to radiation, reproductive history (age at menarche and first pregnancy), tobacco use, and postmenopausal hormone therapy are all variables that raise the risk of breast cancer[4].

BRCA 1, BRCA 2, TP53, and CHEK2 are the most well-known susceptibility genes for family breast cancer. They're all tumour suppressor genes that play important roles in DNA repair and genome integrity. The complete absence of these functions and proteins results in a mutator phenotype and a higher proclivity for accumulating genetic damage, which accelerates cancer development[1].

Breast cancer patients are frequently screened for hormone receptors such as oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2/neu)[5]. The presence or lack of hormone receptors ER and PR, as well as HER2, divides breast cancer into four molecular subgroups. It aids in the prognosis of the tumour and also in the treatment plan[6].

The ER controls the proliferation of both neoplastic and non-neoplastic breast epithelial cells. Intracellular PR, interacts with progesterone to influence the transcription of target genes. HER-2 is a vital marker for assessing the success of targeted therapy and determining prognosis[7].

ER-positive, PR-positive, and HER2-positive malignancies are known as triple-positive cancers. Hormone therapies as well as HER2-targeting medications can be used to treat these tumours. Triple-negative breast cancer (TNBC) cells lack ER and PR receptors and do not produce any or much of the HER2 protein. TNBC is a breast cancer subtype that accounts for up to 24% of all newly diagnosed cases[8] and is more common in women under the age of 40. Hormone therapy and drugs that target HER2 are ineffective in treating these tumours because the cancer cells lack hormone receptors and HER2[9].They are more likely to be larger and of higher grade, have a greater incidence of axillary lymph node metastasis, and have a lower overall survival rate than other forms of breast cancer[5].

The androgen receptor (AR) is emerging biomarker which is a nuclear steroid hormone receptor that is structurally, functionally, and topographically similar to ER and PR[10]. AR has been found to be expressed in 70–90% of breast carcinomas and to play a critical role in the pathogenesis and advancement of breast cancer. Higher levels of AR expression have been associated to an older age at diagnosis, higher ER or PR expression, lower nuclear grades, smaller tumor size, and a lower risk of relapse and death. AR is found in 60–70% of breast cancers, regardless of oestrogen status, and 20–32 % of TNBC patients[11]. TNBC tumors that lack AR expression are referred to as "Quadruple-Negative Breast Cancers (QNBCs)" and may represent a subset of patients with a poorer overall prognosis and a unique molecular signature when compared to AR-positive TNBCs Detecting AR expression in TNBCs and elucidating the AR's role in carcinogenesis could lead to the identification of a target or targets for future therapeutics[12].

Present study aims to evaluate the role of Androgen receptors in carcinoma breast by immunohistochemistry and its comparison with Triple Negative Breast Carcinoma (ER, PR and HER2). This research could provide useful diagnostic and prognostic information to supplement clinical characteristics in order to recommend the best treatment option.

MATERIAL AND METHODS

This study is an observational, cross-sectional and comparative type of study and was carried out in a span of three years (1st Jan 2021 to 1st jan 2024) in Histopathology division of Department of Pathology, Jaipur national university institute for medical science and research centre ,jaipur. The study was conducted on 80 surgically resected breast specimens.

After describing the gross features resected breast were fixed with 10% formalin for 12-24 hours. After routine processing histopathological section was stained by H and E stain. Tumour grading were done by modified Bloom Richardson grading system (Nottingham). Immunostaining were done for ER, PR, Her 2 and AR markers and scored according to nuclear intensity or receptor protein. Then correlation of AR immunoreactivity with existing parameters, such histological grade, stage, ER, PR and HER-2 status was done.Informed consent were taken from patients.

Statistical Analysis: Data was collected and entered simultaneously in SPSS version 23 and coded appropriately. The data was analysed using statistical tests. Descriptive statistics were calculated to summarize the sample characteristics in terms of frequency and percentage. Graphs and Charts were made. Analytical and inferential analysis was done. Chi square test was applied to find association between dependent and independent variables. Continuous data was tested for significance by t-test or ANOVA.

Logistic regression analysis was applied between dependent variable and other independent variables. Significant was set at standard 0.05.

Classifications and Grading System Used:

- **Breast Carcinoma Molecular Sub-Types**

Luminal A	ER +ve and/or PR+ve and HER-2 –ve, Ki67<14%
Luminal B	ER +ve and/or PR+ve and HER-2 +ve or -ve, Ki67=14%
HER-2 Over expressing	ER –ve, PR –ve and HER-2 +ve
Basal-like	ER –ve, PR –ve and HER-2 –ve, CK5/6 and/or CK14+
Normal like	Negative for all five markers

- **Allred (quick) scoring system for oestrogen and progesterone receptor**

Proportion Score	
SCORE	**percentage of Stained Cells**
0	No staining
1	≤ 1 nuclei staining
2	1-10 nuclei staining
3	11-33 nuclei staining
4	34-66 nuclei staining
5	67-100 nuclei staining
Intensity Score	
Score	**Intensity of staining**
0	Negative
1	Weak
2	Intermediate
3	Strong
Allred Scoring= Proportion Score + Intensity Score	
Total Allred Score	**Effect of Hormone Therapy**
0-1	No effect
2-3	Small (20%) chance of effect
4-6	Moderate (50%) chance of benefit
7-8	Good (75%) chance of benefit

- **The IHC test gives a score of 0 to 3+ that measure the amount of Her2 receptor protein on the surface of cells in breast cancer tissue sample**

Score	Staining Pattern	HER2 Assessment
0 to 1+	No or faint staining detected less than 10% of tumour cells.	Her2 negative
2+	A weak to moderate staining observed in more than10% of tumour cells	Borderline
3+	Complete staining is seen in more than 30% of tumour cells	Her2 positive

- **Androgen Receptor expression is semi-quantitative, according to percentage of cell showing nuclear positivity:**

Score	Expression (%)
0	0% expression
1+	1-29% expression
2+	30-69% expression
3+	>70% expression

Androgen receptor will be considered as positive when score ≥1, and negative when score is 0.

RESULTS AND DISCUSSIONS

In the present study, total 80 surgically resected breast specimens were examined grossly and Microscopically. The result of histopathology and IHC were carefully noted, interpreted, and recorded in tabular form.In present study, we were found that among 66.3% of cases had grade II BR Score, 22.5% of cases had grade III BR Score and least 11.3% of cases had Grade I BR Score. Mean BR score was 7, with minimum score of 3 and maximum score of 9 (Table 1).

Hormone receptor status by IHC was obtaind and found that total 48.8% of cases were ER positive status, 42.5% of cases had PR positive status, 23.8 % of cases had Her2 positive status and 65 % of cases had AR positive status and also found that majority (36.3%) were luminal A (ER positive, PR positive and Her 2 negative), Her 2 over-expressing (ER negative, PR negative and Her 2 positive) was seen in 17.5% of cases, 28.8% were TNBC and 17.5% were Luminal B (Table 2).

we analyzed Association of status of Androgen receptor and other factors we found that Androgen receptor was positive for age group >40 years (75%), tumour mass of 2-5 cms (76.9%), Grade II of BR Score (65.4%), stage III of Lymph nodes involvement (44.2%) and Immunophenotype Luminal A (48.1%). Also significant association was found between AR status and Immunophenotype (p=0.00*) and androgen receptor was positive for 89.7%, 82.4% and 52.6% of ER, PR and Her 2 positive cases. Significant association was found between AR status and ER/PR status (p<0.05) (Table 3 and 4 and Fig. 1-7).

Table 1: Distribution of Cases According to Modified Bloom- Richardson Grading System

Modified BR grade	Number of cases	Percentage
I	9	11.3
II	53	66.3
III	18	22.5

Table 2: Distribution of Cases According to ER, PR, HER2 and Ar Status

Parameters	Number of cases	Percentage
ER		
Positive	39	48.8
Negative	41	51.3
PR		
Positive	34	42.5
Negative	46	57.5
Her 2		
Positive	19	23.8
Negative	61	76.3
AR		
Positive	52	65.0
Negative	28	35.0

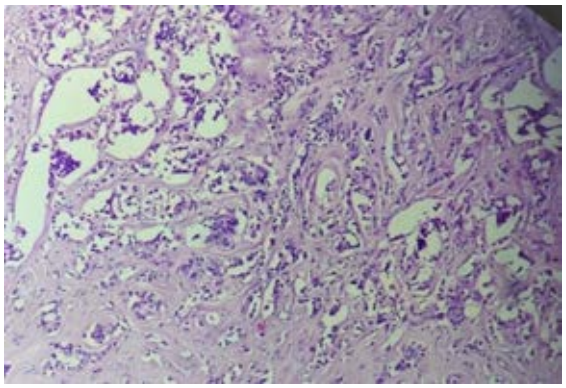

Fig. 1: High power showing minimal variation in cells size, minimal nuclear pleomorphism with rare mitotic activity
IDC of Breast, BR score- 5 BR Grade I (40X, H & E stain)

Table 3: Shows the Association of Status of Androgen Receptor and Other Factors

	AR					
	Positive		Negative			
	Number of cases	Percentage	Number of cases	Percentage	Chi-square	Sig.
Age Group (years)						
<40	13	25.0%	9	32.1%	2.892	.235
41-50	16	30.8%	12	42.9%		
>50	23	44.2%	7	25.0%		
Age Group (years)						
<40	13	25.0%	9	32.1%	.466	.495
>40	39	75.0%	19	67.9%		
Tumour Mass (cm)						
<2 cms	1	1.9%	2	7.1%	1.645	.439[a]
2-5 cms	40	76.9%	19	67.9%		
>5 cms	11	21.2%	7	25.0%		
BR grade						
I	6	11.5%	3	10.7%	.050	.975
II	34	65.4%	19	67.9%		
III	12	23.1%	6	21.4%		
LN stage						
I	16	30.8%	9	32.1%	.204	.903
II	13	25.0%	8	28.6%		
III	23	44.2%	11	39.3%		
Immuno-phenotype						
Her 2 enriched	6	11.5%	8	28.6%	18.157	0.00*
Luminal A	25	48.1%	4	14.3%		
Luminal B	12	23.1%	2	7.1%		
TNBC	9	17.3%	14	50.0%		

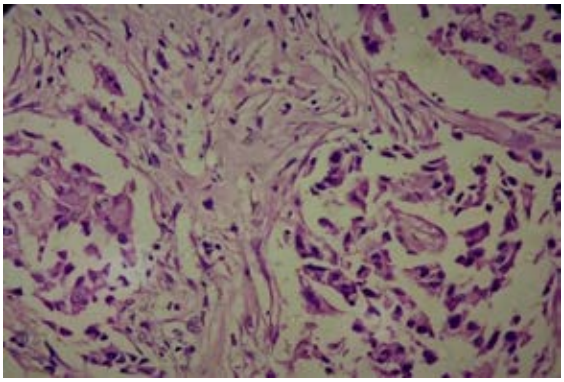

Fig. 2: High power showing increase variation in cells size, nuclear pleomorphism with mitotic activity
IDC of Breast, BR score-7 BR Grade II (40X, H & E stain)

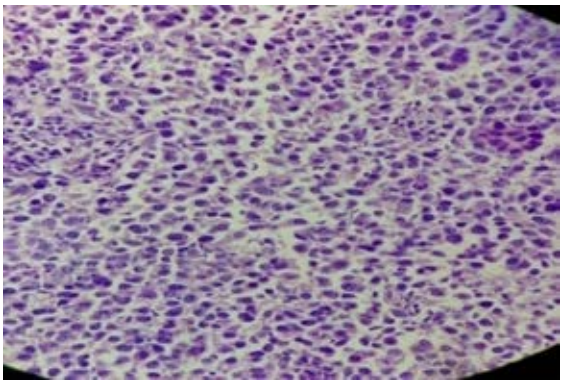

Fig. 3: High power showing marked variation in cells size, nuclear pleomorphism with hyperchromatic nuclei showing coarse chromatin, prominent nucleoli and numerous mitotic activity
IDC of Breast, BR score- 9 BR Grade III (40X, H & E stain)

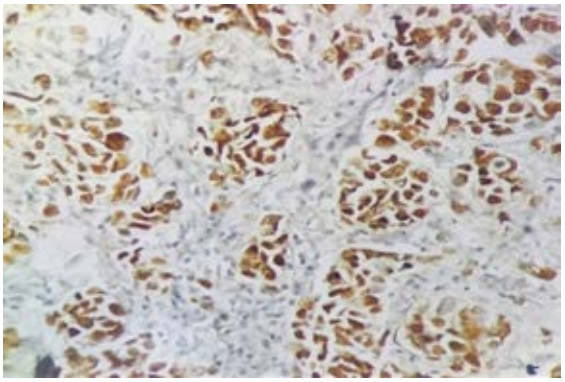

Fig. 4: Sections stained with immunochemical stain (ER) shows 67-100% nuclei suggesting proportion score 5 show strong intensity suggesting intensity score 3. So Allred score: 8

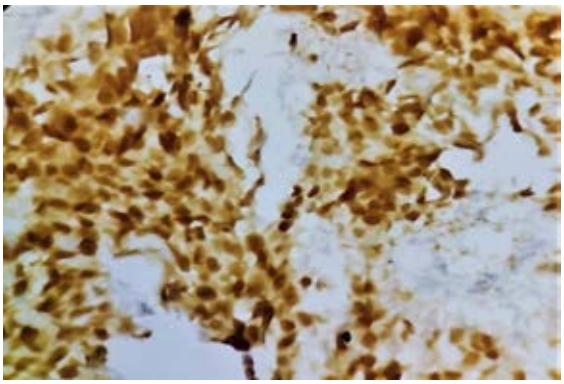

Fig. 5: Sections stained with immunochemical stain (PR) shows 67-100% nuclei suggesting proportion score 5 show strong intensity suggesting intensity score 3. So Allred score: 8

DISCUSSION

Medical advances have made carcinoma of breast curable which was thought to be an irremediable condition earlier.Chances of survival have increased due to the remarkable advances in the screening methods, early diagnosis, and innovations in treatments. Breast malignancy is a hormone dependent tumor. Endocrine therapy is very helpful in those tumors which do express the estrogen, progesterone, Her2 and Androgen Receptor. Hormone receptor analysis is prerequisite now a day before starting adjuvant therapy since it is easier to perform and it also has higher sensitivity.

In present study mean age of the study participants was found to be 50 years. Similar results were reported by Harshima *et al.*, where mean age of the study participants was found to be 55.46 years [12]. It was found that 50% of the participants belongs to 41-60 years of age. 27.5% of cases belongs to 20-40 years of age and remaining 22.5% of cases belongs to 61-80 years of age. Similar to our findings Sandeep *et al*. reported that majority of the participants were >40 years of age (71%)[13].

In present study mean Bloom Richardson grading system score was 7, 66.3% of cases had grade II BR Score, 22.5% of cases had grade III BR Score and least 11.3% of cases had Grade I BR Score. Our finding were in contrast with findings of Sandeep et al and BA *et al* but similar to that of Nikhra *et al*.[14], Villasamy *et al*, H\Geethmala *et al* and BA *et al*.

The assessment of oestrogen receptor, progesterone receptor, and HER2/neu receptor status has become a standard routine procedure in the management of breast cancer, owing to the growing availability of IHC testing facilities and lower costs. The prognosis and response to therapy are determined by the disease's molecular heterogeneity. In present study, majority (48.8%) of cases had ER positive status, 42.5% of cases had PR positive status and 23.8 % of cases had Her2 positive status. Breast carcinoma with her-2 expression were less. Study done by Ahmed et al, who studied hormonal expression in Yemeni women found ER,

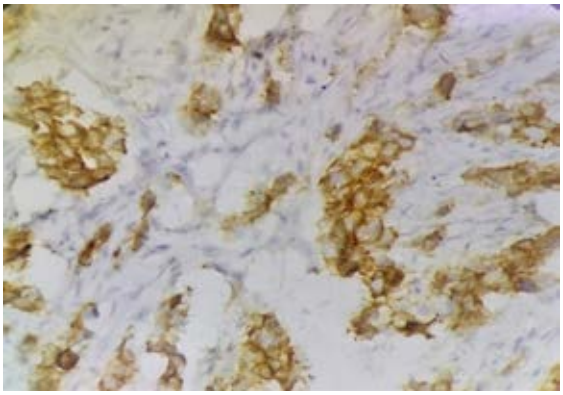

Fig. 6: Sections stained with immunochemical stain (HER2) shows intense staining in more than 30% of tumor cells suggesting score 3 and positive for Her2 stain

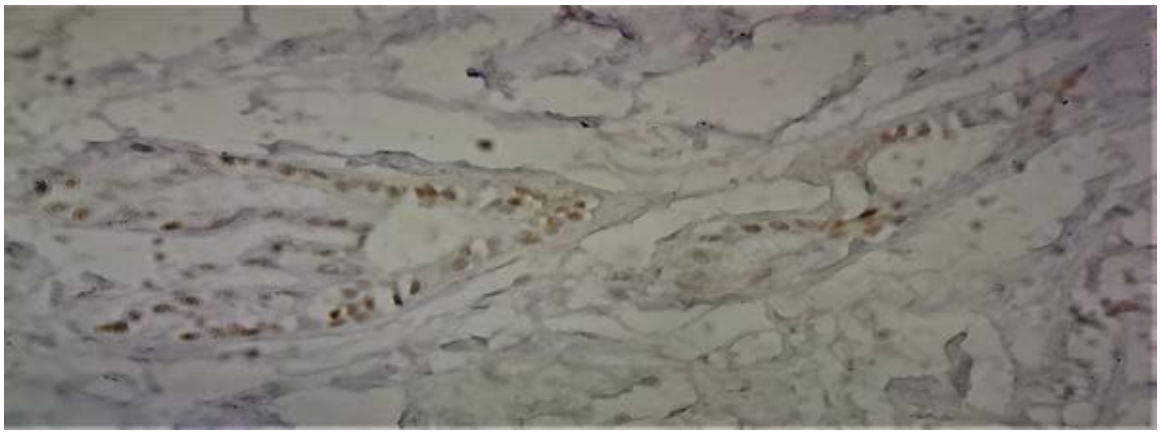

Fig. 7: Sections stained with immunochemical stain (AR), AR expression was semiquantitatively analyzed according to the percentage of cells showing nucleus positivity shows nucleus staining in 30-70% of tumor cells suggesting score 2+ and positive for AR stain

Table 4: Association of Presence of Androgen Receptor and Other Receptors

	ER				PR				Her 2			
	Positive		Negative		Positive		Negative		Positive		Negative	
AR Status	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Positive	35	89.7%	17	41.5%	28	82.4%	24	52.2%	10	52.6%	42	68.9%
Negative	4	10.3%	24	58.5%	6	17.6%	22	47.8%	9	47.4%	19	31.1%
Chi-square	20.479				7.827				1.676			
Sig.	**0.000***				**0.005***				**0.196**			

PR, HER-2/neu in 43.8%, 27%, and 30.6% patients, respectively. Study done by Faheem et al among Pakistani women reported ER, PR, and HER2/neu positivity in 62.2%, 60.1%, and 38.9% patients, respectively. Similar results were reported by Vilasasamy et al and Rao et al. Nikhra *et al*.[14] reported Majority of the study participants with PR positivity.

In present study we did not find any association with age of patient and their tumour expression of ER, PR and Her-2 which are in line with the results reported by Rao *et al*[15].

In present study distribution of immunophenotype shows that, majority (36.3%) were luminal A (Hormone responsive), Her 2 over-expressing (ER negative, PR negative and Her 2 positive) was seen in 17.5% of cases and 17.5% were Luminal B. The percentage of triple negative breast cancer (TNBC) defined as lack of expression of all three receptors, and are associated with poor disease prognosis. 28.8% cases in this study were TNBC. Similar results were reported by Sandeep *et al*, Reddy *et al*, Harshima *et al* and Villasamy *et al*. Ghosh *et al*. have reported TNBC phenotype in 29.8% patients and 32.5% of TNBC was reported by Nikhra *et al*[14]. Slightly lower incidence was reported by BA *et al* (19%), Patnayak *et al* (22%) and Krishnmurthy *et al* (18%)

AR was expressed across all IHC molecular subtypes, with 48.1% of luminal A, 11.5% of HER2 enriched and 17.3% of TNBC subtypes showing AR-positivity. Similar results were reported by Harshima et al, where authors reported 50.6% of luminal, 29.4% of HER2 and 21.6% of TNBC subtypes showing AR-positivity. Statistically significant association was found between AR positivity and IHC molecular Subtypes which is in line with results reported by Harshima et al. Vellaisamy et al in their study reported among luminal A, luminal B, Her 2 overexpression, and triple negative cancers, the rates of AR expression were as follows: 69%, 100%, 57%, and 23%, respectively. Harshima et al also reported association between AR status and IHC molecular subtype

CONCLUSION

The percentage of breast carcinoma patients worldwide is 27.7% of all new cases of cancers. Among these, the patients with ER, PR and HER2 positive cases are 72.2% of all breast carcinoma. the standard tharapy is used for treatment of these patients. This standard therapy is not applicable for patients with ER, PR and HER2 negative. Thus it become a necessity to asseess the Androgen Receptor sensitivity in such cases , which are 17.3% of all breast carcinoma, to promote Anti-androgen therapy for better prognosis and survival of the patients , who have not responded to the standard targeted therapy and were left as untreated earlier with being aggressive in nature.

REFERENCES

1. Vinay, K., A. Kumar, Abul. C.A and Jon, 2017. Robbins and Cotran. Pathologic Basis of Diseases. 10 Edn., Kumar A, ISBN-13: 9780323609937,
2. WHO., 2021. Breast Cancer., https:/ /www .who. int/ news-room/fact-sheets/detail/breast-cancer
3. BC., 2022. Latest Statistics of breast cancer in India- a rising trend in younger women., https://www.breastcancerindia.net/statistics/trends.html#:~:text=69%25%20of%20the%20patients%20were,is%20a%20very%20disturbing%20trend.
4. 2020. Government of India., https://www.india.gov.in/
5. Astvatsaturyan, K., Y. Yue, A.E. Walts and S. Bose, 2018. Androgen receptor positive triple negative breast cancer: Clinicopathologic, prognostic and predictive features. Plos. One., 13: 1-16.
6. Iqbal, B. and A. Buch, 2016. Hormone receptor (er, pr, her2/neu) status and proliferation index marker (ki-67) in breast cancers: Their onco-pathological correlation, shortcomings and future trends. Med. J. Dr. D.Y. Patil Uni., 9: 674-679.
7. Jin, Y.H., Q.F. Hua, J.J. Zheng, X.H. Ma and T.X. Chen *et al*., 2018. Diagnostic value of er, pr, fr and her-2-targeted molecular probes for magnetic resonance imaging in patients with breast cancer. Cell. Physiol. Biochem., 49: 271-281.
8. Marotti, J.D., F.B. de Abreu, W.A. Wells and G.J. Tsongalis, 2017. Triple-negative breast cancer. Am. J. Pathol., 187: 2133-2138.
9. 2016. American cancer society. understanding a breast cancer diagnosis, Atlanta
10. Bhattarai, S., G. Saini, K. Gogineni and R. Aneja, 2020. Quadruple-negative breast cancer: Novel implications for a new disease. Breast. Cancer. Res., 22: 1-11.
11. Zakaria, F., N. El-Mashad and D. Mohamed, 2016. Androgen receptor expression as a prognostic and predictive marker in triple-negative breast cancer patients. Alexandria. J. Med., 52: 131-140.
12. McGhan, L.J., A.E. McCullough, C.A. Protheroe, A.C. Dueck and J.J. Lee et al., 2013. Androgen receptor-positive triple negative breast cancer: A unique breast cancer subtype. Ann. Surg. Oncol., 21: 361-367.
13. Bhaskar, S., K. Rastogi, S. Gupta, A. Jindal, A.R. Bhatnagar and S. Jain, 2019. A study of hormone receptor status in breast carcinoma and use of her2-targeted therapy in a tertiary care center of India. Indian. J. Med. Paediatr. Oncol., 40: 54-60.
14. Pawan, N., 2014. Study of ER (estrogen receptor), PR (progesterone receptor) & HER- 2/NEU (human epidermal growth factor receptor. Int. J. Biomed. Adv. Res., 6: 275-278.
15. Rao, C., J. Shetty and H.K. Prasad, 2013. Morphological profile and receptor status in breast carcinoma: An institutional study. J. Cancer. Res. Ther., 9: 44-49.