



Prevalence of androgen receptor positivity in triple negative breast cancers: clinico-pathological significance

Authors

Debahuti Mohapatra, Debasmita Das*, Pranita Mohanty, Rajashree Tripathy
Department of Pathology, IMS and SUM hospital, Siksha "O" Anusandhan University,
K8, Kalinganagar, Bhubaneswar-751003, Odisha, India

*Corresponding Author

Dr Debasmita Das

P.G Student, Dept. of pathology, IMS & SUM Hospital

Email: dasdiya64@gmail.com, Cell: 9620814629

Abstract

A unique subgroup of breast cancer, the Triple negative breast carcinoma (TNBC) is on rising trend. TNBC occurrence is around 15%-20% of all breast cancers. They are defined by absence of target therapy and prognostically worse as far as size, morphology, grading and staging are concerned. Thus, recent advent of androgen receptor (AR) sensitive target in some of the TNBC, which though are prognostically bad but has initiated target therapy successfully but still is under study. It is a sign of optimism for the oncologist for the treatment of incurable types of TNBCs. A study was done at IMS and SUM hospital, Bhubaneswar on 110 cases of breast carcinoma, among which 24 cases were TNBC. These included high grade infiltrating duct carcinoma (IDC-NST), metaplastic carcinomas, medullary carcinomas, neuro-endocrine carcinoma, apocrine carcinoma and acinic cell carcinoma. The tumors were designated as triple negative based on ER, PR, HER 2/neu staining. The proliferative index (Ki-67) and as well as AR staining were additional done, which showed 16.66% cases of AR positivity and most of which showed high proliferative index more than 21%.

Keywords: TNBC, Androgen receptor, Breast carcinoma.

Introduction

Breast cancer is the second most common cancer in women which accounts for 23% of all female cancers, posing a major public health problem worldwide. The expression of AR in primary breast carcinoma is 60%-70% i.e. in infiltrating ductal carcinoma (IDC) non-basal type, ductal carcinoma in situ (DCIS), lobular carcinoma, BRCA positive cases and Paget's disease^[1]. The expression of AR in ER negative tumour is 30%, whereas the AR positivity in TNBC is 20%^[2-5].

Triple negative breast cancer (TNBC) is defined as negative for ER, PR (<1% of tumour cell nuclei)^[8] and Her2/neu (score of 0,1+ or 2+) comprising of high grade IDC, metaplastic carcinoma, medullary carcinoma, neuroendocrine carcinoma and apocrine carcinoma. TNBC occurrence is around 15% – 20% of all breast cancers. This is not only a prognostic factor but also a predictive factor. Thus, it may reflect the natural history of tumour (prognostic factor) and the likely response to the treatment (predictive

factor)^[2-3]. The AR positivity decreases in the end-staged metastatic disease showing an importance of AR targeted therapy at an earlier stage^[2-5].

Materials and Methods

The total number of cases of breast carcinoma included in the study from eastern Indian population admitted in IMS and SUM hospital were 110 between the period of June 2015 to September 2018. Majority i.e. 72.22% belonged to 50-60 years of age followed by 27.78% belonged to <50 years of age. Lumpectomy or mastectomy was done. Following which IHC for ER, PR, Her2/neu, AR, CK 5/6, p63 and Ki-67 was performed.

Cell proliferation (Ki-67) was assessed by counting at least 500 tumor cell nuclei (depending upon the availability of tumor) and graded as low (<11%), intermediate (11–20%), and high (>21%)^[6].

IHC for CK 5/6 was done using antibodies D5/16 B4 for CK5/6 (Dako, Glostrup, Denmark) and if the staining was found in >50% of tumor cells, it was interpreted as positive and if <50% of tumor cells, it was interpreted as negative, as previously reported^[7].

For the IHC staining for AR, antigen retrieval was performed as follows: five micron sections were deparaffinised and rehydrated to deionized water. They were heated in citrate buffer (pH 6.0) using an electric pressure cooker for three minutes at 12-15 pounds per square inch (PSI) at approximately 120 degrees Celsius. They were then cooled for ten minutes prior to immunostaining. All slides were loaded onto an automated system (DAKO Autostainer plus, DAKO) and exposed to 3% hydrogen peroxide for 5 minutes, incubated with primary antibody for thirty minutes, with labelled polymer for thirty minutes, 3,3'-diaminobenzidine (DAB) as chromogen for five minutes, and then with hematoxylin as counterstain for five minutes. These incubations are performed at room temperature and between incubations, sections are washed with Tris-buffered saline (TBS). The gradation was done for the AR positive cases as

low (<10% of the nuclei showing positivity), medium (about 10% moderate positivity and high > 10% strong positivity)

All the cases were evaluated independently by two pathologists. The consensus results were recorded and the discordances were resolved by review and discussion.

Results

The 110 women ranged in age from 28-70 (median, mean) years at the time of diagnosis. The TNBC cases were 24 cases which included high grade IDC (NOS) i.e. 12 cases, medullary carcinoma. 3 cases, metaplastic carcinoma i.e. 4 cases, neuroendocrine carcinoma i.e. 2 cases, acinic cell carcinoma (1 case) and apocrine carcinoma (2 cases). The gradation was done for the AR positive cases as low (<10% of the nuclei showing positivity), medium (about 10% of the nuclei) and high (>10 % of the nuclei) . Amongst these cases, high positivity AR status was found in post-menopausal age group (>50 years) and low positivity in pre and peri-menopausal age groups (<50 years). Tumour size varied from 0.5cm to 10 cm. The maximum occurrence of AR positivity was seen in breast tumour of <3cm in size. The variants of tumours showing AR positivity were high grade IDC (NOS) [n=2, 16%] (fig.1a & 1b) and apocrine carcinoma [n=2, 100%] (fig.2a & 2b). The metaplastic carcinoma, medullary carcinoma, acinic cell carcinoma and neuroendocrine carcinoma were AR negative (fig.3 & 4). One case of TNBC was found to be basal type showing strong CK5/6 positivity and was found to be AR negative. Strong AR positivity was found in 9% cases whereas, medium and low positivity was seen in 4 and 1% cases respectively (Fig 5).

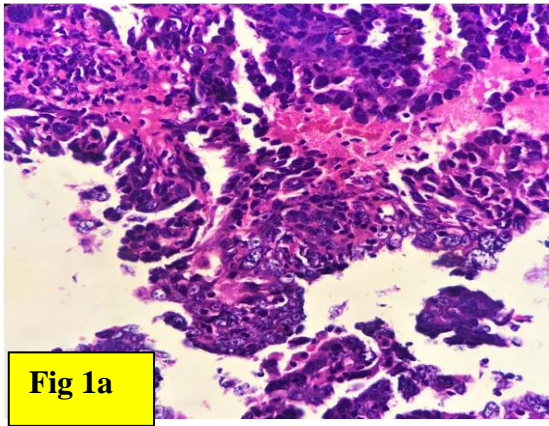


Fig 1a

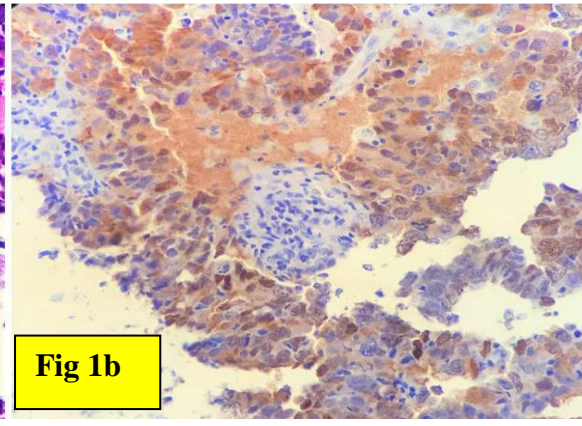


Fig 1b

Fig 1a Microphotograph of IDC-NST (MBR grade III) showing neoplastic cells in papillae (H&E x 400), **1b** AR moderate positive (IHC X 400)

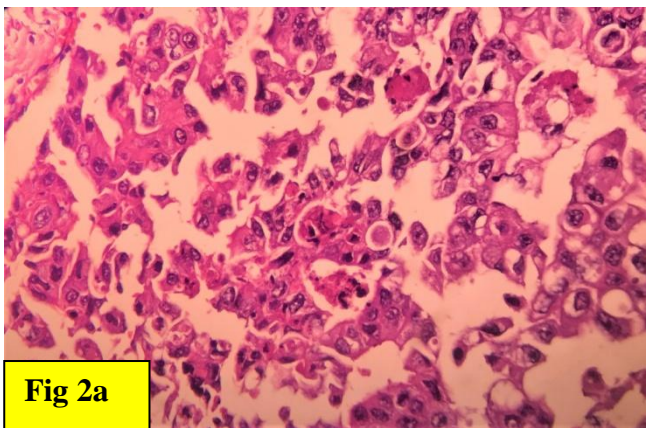


Fig 2a

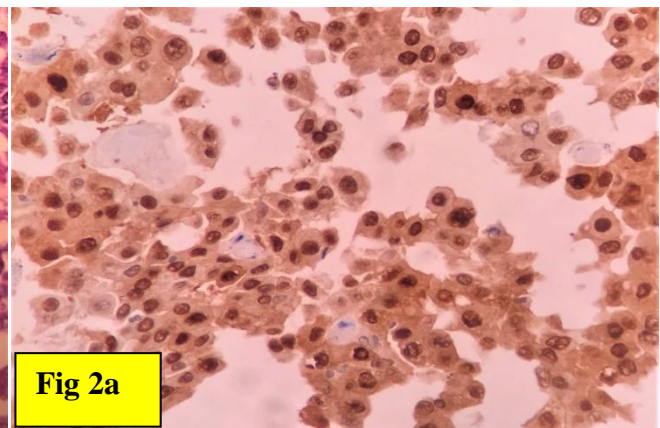


Fig 2a

Fig 2a Microphotograph of apocrine carcinoma showing ductal epithelial cells showing apocrine change and luminal secretion (H & E X400), **Fig 2b** Strong AR positivity. (IHC X 400)

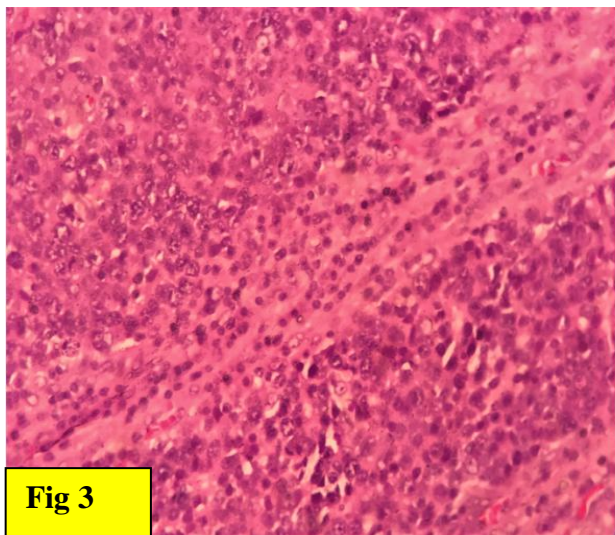


Fig 3

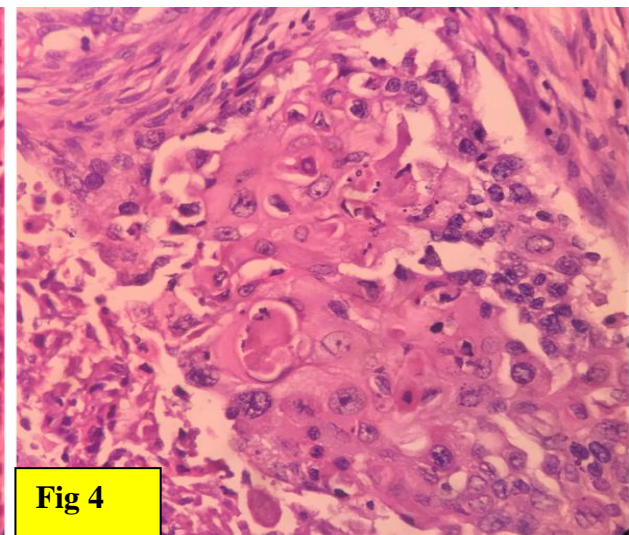


Fig 4

Fig 3 microphotograph of medullary carcinoma showing nests of neoplastic cells intervened by lymphocytes (H& E X 400)

Fig 4 Microphotograph of Metaplastic (squamous) carcinoma of breast showing ductal cells with neoplastic squamous cells. (H & E 400)

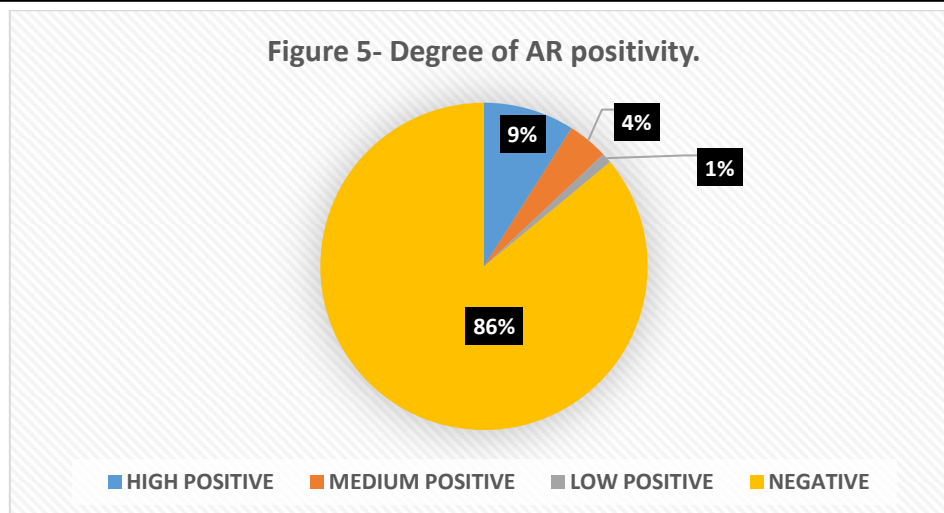


Fig 5

Positive AR status was found to be more associated with histologic grade III (75%) tumours, whereas only one case of histologic grade II (25%) showed moderate positivity. 13 out of 24 cases of TNBC showed metastasis to axillary lymphnode. In our study, maximum AR

positivity was seen in 1-3 lymph nodes (75%). Whereas 4-9 lymph node positivity was seen in 25% cases. AR positivity status was more commonly found in stage III cases. 83.33% of AR positive tumour showed high proliferative index (>21%) (Table 1).

Table 1 Showing clinico-pathological correlation between AR positivity in various types of TNBC

VARIABLES	TOTAL TNBC CASES (%)	AR +VE CASES (%)	AR -VE CASES (%)
TOTAL NUMBER OF PATIENTS (%)	24	4	20
AGE (YEARS)			
<50	06	00	06
50-70	16	03	13
>70	02	01	01
TUMOUR SIZE			
<2cm	11	00	11
2cm-5cm	09	04	05
> 5cm	04	00	04
TYPES OF TUMOUR			
1. High grade IDC (non-basal like)	10	02	10
2. High grade IDC (basal type)	02	00	00
3. Apocrine CA	02	02	00
4. Medullary CA	03	00	03
5. Metaplastic CA	04	00	04
6. Neuroendocrine	02	00	02
7. Acinic cell CA	01	00	01
GRADE			
I	02	00	02
II	06	01	05
III	16	03	13
LYMPH NODE			
pN0	10	00	10
pN1(1-3)	09	04	05
pN2(4-9)	03	00	03
pN3(10 and >)	01	00	01
pNx	01	00	01
STAGE			
I	00	00	00
II	05	00	05
III	16	04	12
IV	03	00	03
NA	00	00	00
Ki-67			
≤10 %	01	00	01
11%-21%	03	00	03
> 21%	20	04	16

Discussion

Breast cancer cases with ER positivity is about 70 %, HER 2 neu positivity is about 15 % and TNBC is about 15%^[9,10]. The occurrence of AR in TNBC has recently drawn attention. One third of ER negative cases and one third of BRCA positive cases with high grade invasive cancers have AR expression^[11,12].

Androgens are necessary precursors of estrogen synthesis in the ovary. Androgens are secreted by both ovaries and adrenals. The main androgen is secreted as Androgen dehydro epiandrosterone sulfate and non-sulphated forms (DHEAS or DHEA respectively) inhibits growth of breast cancer. Androstendione is the other precursor of testosterone converting the low potency to high potency form, expressed in many cell types such as stromal breast cancer cells and epithelial cells^[13]. Studies have shown increased testosterone levels and low progesterone levels have increased risk for breast cancer^[14].

Androgen has been described as a potential tumor suppressor in ER-positive breast cancers with its anti-proliferative effect presumed to result from cross talk between steroid receptor signaling pathways^[15]. However, studies investigating AR in TNBC have reported conflicting results. For example, Birell et al. noted that AR had a proliferative effect in ER and PR negative cell lines^[16] which was confirmed by Garay et al. and by Doanne et al., who raised the possibility of targeting the androgen pathway^[17,18].

AR positive TNBCs were found in 16.66% cases of TNBCs in our study, majority of which occurred in post menopausal patients (>50 year). The average tumor size was 2-5 centimetres. Luo et al found that AR was expressed in 28% TNBC and correlated significantly with postmenopausal status. The common grading was grade III (modified Bloom Richardson grading) showing 1-3 lymphnode metastasis. Rakha et al. reported that AR positivity was associated with higher grade. The common tumor showing AR positivity were Apocrine carcinoma and High grade IDC-NST, non-basal type. The basal type of IDC-NST was

found to be negative for AR receptor compatible with Bryan et al^[4].

Conclusion

AR-positive TNBC may represent a breast cancer subtype with unique features that may be amenable to treatment with alternative targeted therapies. The AR positive tumours are more prone for recurrence and metastasis. Such metastatic tumours usually yield AR negativity. Thus the target therapy at an early stage is extremely important in all cases of AR positive TNBCs.

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