International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Correlation of Expression of Androgen Receptors and P16 in Triple Negative Breast Carcinoma - A Five Year Retrospective And Prospective Study

Dr. Ajeet Kumar Prajapati[1], Prof. Veena Maheshwari[2], Prof. NishatAfroz[3], Dr. Ankita Parashar[4]

[1]Postgraduate, Department of Pathology, Jawaharlal Nehru Medical College and Hospital (JNMCH), Aligarh, India.
[2,3]Professor, Department of Pathology, Jawaharlal Nehru Medical College and Hospital (JNMCH), Aligarh, India.
[4]Senior resident, Department of Pathology, Jawaharlal Nehru Medical College and Hospital (JNMCH), Aligarh, India.

Abstract:

AIM AND OBJECTIVE

Triple negative breast carcinoma (TNBC) is an aggressive breast carcinoma, lacking estrogen receptor (ER),progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2neu receptor) amplification, thereby, unresponsive to conventional hormonal therapy. The aim of present study is to examine androgen receptor (AR) and p16 expression in TNBC cases and explore its clinical significance in view of potential AR and p16 targeted TNBC therapy.

MATERIAL AND METHOD

A total 94 TNBC patients were included in this 5 years (3 years retrospective and 2 years prospective) study, conducted at Department of Pathology, JNMCH, Aligarh. Immunohistochemical stains for AR and p16 were performed and their relationship with TNBC clinicopathological data were analyzed. Positive AR expression was defined as ≥1% nuclear staining wheras positive p16 expression was defined as score of ≥1which in turn calculated as product of intensity score and extent of positivity score of cytoplasmic and nuclear stained cells.

RESULT

Out of 94 TNBC patients, 92 cases were of invasive carcinoma (NST) and 2 cases were invasive lobular carcinoma.AR was expressed in 38/94 (40.4%) cases, all of which were of invasive carcinoma (NST) type. We observed higher expression rate in postmenopausal women i.e.43.2% (16/37) cases and in patients with age ≥60 years i.e. 66.7% (8/12) cases. Statistical analysis showed significant association of AR + TNBC cases with larger tumor size (p= 0.017412) and lymph node metastases (p= 0.033119). Higher expression rate was found in lower grade and stage III, however statistically insignificant.p16 protein was positively expressed in 72.3% (68/94) of the total TNBC cases. We observed higher expression rate in premenopausal women i.e.78.9%(48/57) cases and in patients with age 40-49 years i.e. 84.4% (27/32) . Significantly higher p16 expression rate was found in grade II (90%) followed by grade I (66.7%) and grade III (65%) (p= 0.04).

CONCLUSION
TNBC was more common in older age group and had a higher propensity for lymph node metastases. AR positive and p16 positive TNBC cases may represent a breast cancer subtype with unique features that may be amenable to treatment with alternative targeted therapy.

KEYWORDS: Androgen receptor, p16, Triple negative breast carcinoma, TNBC

INTRODUCTION

Breast carcinoma have spectrum of characteristics ranging from well differentiated homogenous to mixed heterogeneous entities. Studies have shown that prognosis depends on the biological or molecular subtypes of the carcinoma. That's why; immunohistochemistry plays an important role in its final diagnosis. Several molecular expressions in breast carcinoma as well as their application have been studied. Estrogen Receptors (ER), Progesterone Receptors (PR) and Human Epidermal Growth receptor (EGFR-2 or HER 2-neu) markers have been used routinely for identification of various types of luminal cell breast carcinoma. Luminal cell breast carcinoma which does not express the ER, PR and EGFR2 molecules are labeled as triple negative breast carcinoma (TNBC) [1]. Since TNBC cases don't have actionable receptors for therapeutic target, its treatment is hampered. Lacking of targeted therapy and poor prognosis as warranted a major effort to discover specific and actionable molecular targets for treating TNBC cases. Molecular studies have also led to identification of certain targetable features such expression of androgen receptor and p16 and several other genomic alterations. However, still it is not clearly established whether these alterations are molecular 'drivers' or not along with their effects in different types of breast carcinoma[2,3]. The objectives of the current study are to examine the prevalence of AR and p16 expression and their utility in triple negative breast carcinoma (TNBC) patients.

MATERIAL AND METHOD

The present study was conducted on 94 patients diagnosed as triple negative breast carcinoma for analyzing the Androgen Receptor and p16 expression in them. The work was undertaken in the Department of Pathology, Jawaharlal Nehru Medical College (JNMC), Aligarh, (UP) from 2017 to 2019. We studied 3 years of retrospective cases and 2 years of prospective cases.

The surgical breast specimens received from The Department of the Surgery, JNMC, Aligarh, were studied under gross examination and characteristics were noted. Required sections were taken as par the standards and after fixation, tissue pieces were processed in Automated Tissue Processor set, LEICAM TP1020 (Germany), for a 24-hour cycle. Processed tissues then transferred from the final wax bath to the disposable embedding cassettes (rectangular moulds), filled with molten paraffin wax to form tissue blocks. After solidification, thin sections of 3- 5 microns thickness were cut using a Rotary Microtome (LEICA RM 2125 RT) followed by immediate floating in a water bath at 60 0C. The sections were mounted onto clean glass slides coated with albumin and stained with routine H&E stain using Harris Hematoxylin and aqueous Eosin[4].

AR Immunostaining was done with ready to use reagents obtained from Thermmoscientific company and p16 immunostaining with ready to use reagent from Biogenexcompany as par their protocols followed by slide examination under microscope.Cervical Carcinoma and Prostate Adenocarcinoma served as positive control for p16 and AR respectively. Absence of primary antibody was taken as negative control for both the immunostains.

Interpretation

IHC scoring of p16 expression will be analyzed according to the intensity as well as percentage of the stained cells showing nuclear as well as cytoplasmic positivity [5]. Score for intensity of the nucleic or cytoplasmic staining - 1 (Weak staining), 2 (Moderate staining) and score 3 (Strong staining). Score for extent of stained cells – 0 (<10%), 1 (10-25%), 2 (26-50%), 3 (51-75%), 4 (> 75%). Final score is obtained by multiplying intensity score with extent of positivity score of stained cells. Minimum and maximum score will be 0 and 12, respectively. p16 expression will be considered positive when score is ≥ 1, and negative when score is 0. Final Score Interpretation - 0 (Negative), 1-4 (Weakly Positive), 5-8 (Moderately Positive) and 9-12 (Strongly Positive).

IHC scoring of AR expression will be analyzed according to the percentage of cells showing nuclear positivity [6]. We have assigned score from 0 to 2+ as follows - 0 % (Negative), 1+ (1% -10% , low positive) and 2+ (>10% ,positive). AR expressionwill be considered positive when ≥ 1% of the cells show nuclear positivity.

Statistical analysis

The statistical analysis was carried out using SPSS software V 20.0, for determining the statistical significance, Student's t and Fisher's exact/ chi square tests were used for continuous and categorical variables, respectively. A p-value of <0.05 was considered to be statistically significant.

RESULTS

Total number of breast carcinoma cases received during 5 years were 434 cases, out of which, on 228 cases, ER, PR and HER2/neu immunomarkers were applied. After IHC application, 94 cases turned out to triple negative, giving triple negative breast carcinoma (TNBC) in 41.2% (94/228) of the breast carcinoma cases. Correlation of TNBC cases with clinicopathological parameters are shown in Table 1.The most common specimen received is mastectomy specimen (93.6%) with breast lump as common clinical presentation. Left breast was affected slightly more (51.1%) with upper outer quadrant involved most commonly (i.e. 59.6%). TNBC was found more commonly in premenopausal women (60.6%) and age group of 40-49 years (34%). Most of them had tumor size between >2-≤ 5 cm (72.3%) and are of invasive carcinoma (NST) type (97.8%) and 2 cases (2.2%) of invasive lobular carcinoma type. We reported grade 3 to be the most common type (65.2%). Majority of TNBC cases (75.5%) were negative for lymphovascular invasion with 62.7% cases associated with LN involvement. N1 was the most common nodal stage among LN positive cases with II B the most common stage (40.3%).

AR were positively expressed in 40.4% of the TNBC cases and all are of invasive carcinoma (NST) type with higher expression rate without significant difference in postmenopausal women (43.2%), age ≥ 60 years(66.7%), grade I tumor (66.7%), T4 stage (66.7%), N3 (100%) and in stage IIIC (100%). AR expression rate was significantly higher in tumors of size > 5 cm (66.7%) (p = 0.0174). LV invasion and LN metastases are significantly higher in AR positive cases with involvement of 39.5 % (p= 0.005) and 78.6% (p= 0.022) of the total AR positive cases respectively (Table 2). AR expression score correlation with grade, primary tumor stage and nodal stage have been summarized in Table 4.

P16 protein was positively expressed in 72.3% of the TNBC cases, all belongs to invasive carcinoma (NST) type. Its expression rate was found to be in premenopausal women (78.9%), age group of 40-49 years (84.4%), smaller TNBC tumor size of ≤ 2 cm (80%), T1 stage (80%), N0 nodal stage (76%) and stage II A (79.2%), however, insignificantly. Significantly higher p16 expression rate was found in grade II (90%) in comparison to other stages (p= 0.04). Lymphovascular invasion as well as lymph node

metastases rate was found to be more in p16 negative cases i.e. 38.5% and 68.4% respectively (Table 3). P16 expression score correlation with grade, primary tumor stage and nodal stage have been summarized in Table 5.

DISCUSSION

Our study reported the TNBC incidence of 41.2% supported by other studies like Nigam and Sood,(2014)[7], Jana et al.,(2014)[8] and Akhtar et al.,(2015)[9] with similar TNBC incidence of 39.4%,46.7% and 43.5% respectively. However, Doval et al., (2015)[10] and Patnayak et al.,(2015) [11] observed lower incidence of 23.8% and 22.7% respectively in their studies. Boyle et al., (2012) [12] from California observed 20% incidence of TNBC.

We reported 60.6% of the TNBC cases were of premenopausal women with similar reporting by Rao et al.,(2013)[13] from south India and Sen et al.,(2012) [14]from east India of 67.4% and 54.1% respectively. However, Akhtar et al., (2015)[9] from west India and Nigam and Sood,(2014)[7] from north India found higher TNBC incidence in postmenopausal group i.e. 58.8% and 54.6% respectively. Our study observed age group of 40 to 49 years to be mostly affected with median age of 45 years with similar finding of median age (46.1 years) and range (41-51 years) in a study in west India by Singh et al., (2014)[15]. However, Jana et al.,(2014)[8] and Boyle et al.,(2012)[12] reported median age of 54.6 years and 57 years respectively indicating higher median age in western world females.. We found the left breast to be slightly more affected i.e. 51.1% as also shown by Dent et al.,(2009)[16] in Toronto Canada (58.3%).However, Suresh et al.,(2013)[17] reported higher TNBC incidence in right breast i.e. 51.9% and bilaterally affecting 0.6%. Also Dent et al.,(2009)[16] showed higher bilateral breast involvement in western females (i.e. 9.5%) as compare to Indian females in studies. Upper outer quadrant was mostly affected site in our study (59.6%) with similar findings of 67% in Bashir et al., (2017)[18] and 34% in Arora et al.,(2019)[19] studies. Tumor size of 2 to 5 cm was the most commonly affected size shown in our study i.e. 72.3% as well as in Nabi et al.,(2015) [20] i.e. 76.2%. However, Akhtar et al.,(2015) observed tumor size of more than 5 cm to be mostly affected(64.7%). Invasive carcinoma (NST) was the most common histo-morphological type (97.8%) of TNBC as described in other Indian studies (Mane et al., 2015[21] and Patnayak et al., 2015[11]) as well as western world study (Pareja et al.,2016)[22]. Most of the TNBC cases were of higher grade with pattern of grade 3(64.5%) > grade 2 (32.3% > grade 1 (3.2%) with 1 cases of carcinoma with medullary like features always considered of high grade. Kim et al., (2017)[23] found the similar result but Rao et al.,(2013)[13] found grade 2 to be the most common grade. We received lymph nodes in 67 cases out of total 94 TNBC cases with 62.7% lymph node involvement as also reported by Nabi et al.,(2015)[20] in their study. However, Singh et al., (2014) and italian study Urru et al.,(2018)[24] found most of the lymph node to be negative. Most of the TNBC cases in our study were of TNM stage II (76.1%) supported by other studies (Reddy et al., 2018[25] and Kim et al., 2017). In contrast, Agarwal et al.,(2015)[26] reported TNM stage III to be the most common stage in 47.5% of the TNBC cases.

We studied AR immune expression in 94 TNBC cases, considering TNBC case with >1% of tumor cells showing AR nuclear staining as AR positive. We reported 40.4% TNBC cases were AR positive in agreement with the Astvatsaturyan et al.,(2018)[27]. However, Liu et al.,(2018)[28] from China showed AR positivity only in 21.8% of the total cases considering the same cut off value for AR positivity. This shows the wide range of AR positivity in TNBC cases attributed to TNBC tumor heterogeneity and a lack of universally accepted standards and analytical protocols for determining the AR positivity. We found 38.6% of the premenopausal women and 43.2% of the postmenopausal women were AR positive without

any significant association (*p value* = 0.6537) as also documented by Liu et al.,(2018)[28]. In contrast, Arora et al.,(2019) [19], found significant association with menopausal status (p= 0.01). We observed AR expression rate be higher in TNBC cases of age group > 60 years (66.67%) followed by 40-49 years (46.9%) without any significant difference (*p value* = 0.174) among different age groups similar to Arora et al.,(2019)[19] study. Astvatsaturyan et al.,(2018)[27] reported mean age of women in the AR positive group was significantly older than that of AR negative group (*p value* = 0.015). It indicates that AR positivity in TNBC cases may increase with age.

We observed the AR expression rate among grade 1 TNBC tumor of 66.7% followed by grade 2 (43.3%) and grade 3(38.3%) without any significance (*p value* = 0.104) and similar pattern reported by McGhan et al.,(2014)[29]. Sunar et al.,(2018) also observed the similar pattern for AR expression rate with significant association between AR expression and grade (*p value* = 0.001), indicating AR expression rate is associated with lower TNBC grade. We found higher intensity of AR expression score in lower grade in comparison to higher grade without any significant association (*p value* = 0.890) (Table 4) in agreement with Arora et al.,(2019)[19]. It indicates that AR might play major role in early developmental stage of the TNBC cancers. We documented a trend of increasing AR expression rate with increasing primary tumor stage i.e. T1(20%)< T2(32.8%) < T3 (56.3%) < T4 (66.7%) without any significant difference among primary tumor stages, supported by similar pattern found in Astvatsaturyan et al.,(2018)[27] study. We found that AR positive TNBC cases were significantly associated with higher rate of lympho vascular invasion (*p value* = 0.0053). McGhan et al.,(2014)[29] also reported similar invasion rate but without any significance. In contrast, Teoh et al.,(2019)[30] from Malasiya, reported lympho vascular invasion rate higher in AR negative TNBC cases without any significant difference (p = 0.056).

In our study, 78.6% of the AR positive TNBC cases were showing metastases to the lymph node(s) which was significantly higher in comparison to 51.3% of the AR negative TNBC cases found to be lymph node positive for tumor cells (*p value*= 0.022) as supported by other studies [McGhan et al.,2014[29](p = 0.03) and Arora et al., 2019[19] (*p value* = 0.013)]. However, Tang et al., (2012)[31] found more lymph node positivity rate in AR negative TNBC cases (88.1%) without any significant differences. We found higher AR expression rate in higher nodal stage without any significant difference among nodal stage. However, Hu et al.,(2011)[6] and Astvatsaturyan et al.,(2018)[27] higher AR expression rate in N0 and N1 respectively indicating variable possibilities of different AR pathways playing role in genesis of TNBC leading to its propensity to get metastasized to lymph node (s). We observed that most of the AR positive cases belonged to stage IIB (39.3%) followed by stage IIA (33.3%) with higher expression rate in stage IIIC (100%) followed by IIIB (66.7%).Overall, most of the AR positive cases were of stage II followed by stage III and I without any association between AR expression and TNM stage (*p value*= 0.272). Similar finding was reported by Sunar et al.,(2018) [32]. Arora et al., 2019 documented most common stage for AR positive TNBC cases to be stage I (56.7%) and highest AR expression rate in stage IIB (66.7%) with no significant association between AR expression and TNM stage (*p value*= 0.59).

We also studied p16 expression in 94 TNBC cases showing 72.3% (68/94) p16 positive cases. Shin et al.,(2015)[33] from South Korea reported 84.9% and whereas Bogina et al.,(2014)[34] reported only 49.6% of the TNBC cases with p16 positivity. This wide range of p16 expression positivity could be explained on the basis of interaction between p53 protein, Rb protein and p16 protein during carcinogenesis and their feedback mechanism. A higher p16 immuno expression rate was observed among premenopausal women (78.9%) than in postmenopausal women (62.1%) without any significant difference (*p value*= 0.07), supported by Abou – Bakr and Eldweny (2013) [35]. We documented higher expression rate in age

group 40-49 years(84.4%) > 30-39 years (80%) without any significant difference with similar findings reported by Bogina et al.,(2014)[34].

We noted significantly higher p16 expression rate among grade 2 TNBC cases (90%) followed by grade 1(66.7%) and grade 3(65%) (*p value* = 0.04).However, Hashmi et al.,(2018) [36]documented highest rate among grade 3 >grade 2. We observed higher p16 expression score among grade 2 TNBC cases supported by Hashmi et al.,(2018)[36]. However, no significant difference was found among score and grade in any study (Table 5). We observed inverse relation of p16 expression rate to the tumor stage i.e. T1 (80%) > T2 (78.7%) > T3 (68.8%) > T4 (41.7%) without any statistical significance (p = 0.068). In contrast, Hashmi et al.,(2018) [36] reported **2018**documenteddirect relationship of higher expression score for p16 with tumor stage i.e. T1 (66.75%) < T2 (71.8%) < T3 (73.8%).This variable p16 expression rate could be due to complex interaction between p16, p53 and Rb proteins. We found higher rate of lympho-vascular invasion in p16 negative TNBC cases i.e. 38.5% of the p16 negative TNBC cases in comparison to lympho-vascular invasion in only 19.1% of the p16 positive TNBC cases without any statistical significant (p= 0.051) supported by Bogina et al.,(2014) [34]. However, Hashmi et al., (2018) observed higher lympho vascular involvement in p16 positive TNBC cases without any significant association. We observed lymph node involvement in 68.4% of the p16 negative cases,higher than found in p16 positive casses (60.4%) without any significant difference (p = 0.54), supported by Shin et al., 2015 [33]. Moreover, we found that the p16 expression rate was higher in N0 (76%) followed by N1 (73.3%), N2 (60%) and N3 (50%) i.e. N0>N1>N2>N3. No statistical difference was found among the different nodal stages (p = 0.704). We noted higher p16 expression intensity in lower nodal stages and observed that 36% of TNBC cases with N0 stage showed strong positivity, moderate positivity was maximally shown by N1 stage and weak positivity maximal in N3 stage without significant difference among N0, N1 and N2/N3 (p = 0.569). This difference with our study could be due to the different molecular pathways playing role in TNBC genesis. One of the limitations to our study was that molecular testing of androgen receptor and p16 was not performed and therefore, we suggest related molecular testing in TNBC cases of our population to establish the mutation status and its correlation with their over expression immuno-histochemically. Also, our data are limited because of the smaller numbers of patients who were not equally distributed between positive and negative results for androgen receptor and p16. A larger sample size and a matched cohort is warranted for better understanding and characterization of role of androgen receptor and p16 in TNBC patients.

CONCLUSION

Androgen positive triple negative breast carcinoma (TNBC) cases was more common in older age and had high propensity for lympho-vascular invasion and lymph node metastases. AR- positive TNBC may represent a subtype of breast carcinoma, with unique features that may be amenable to treatment with alternative targeted therapy. Moreover, high expression of p16 in TNBC suggests a potential role of this biomarker protein in TNBC pathogenesis as well as in developing targeted therapy in p16 positive TNBC patients.

References

1. Bareche Y, Venet D, Ignatiadis M, Aftimos P, Piccart M, Rothe F et al. Unravelling triple-negative breast cancer molecular heterogeneity using an integrative multiomic analysis. Annals of Oncology 2018;29: 895–902.

2. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nature Reviews Clinical Oncology 2016;13(11):674-690.
3. Bohn OL, Fuertes-Camilo M, Navarro L, Saldivar J, Sanchez-Sosa S. p16INK4a expression in basal-like breast carcinoma. International Journal of Clinical and Experimental Pathology 2010;3(6):600-607.
4. Gamble M and Wilson I. The hematoxylins and eosin. Theory and Practice of Histological Techniques 2008;6:121-134.
5. Zhao LH and Liu HG. Immunohistochemical detection and clinicopathological significance of JARID1B/KDM5B and P16 expression in invasive ductal Bibliography 138 carcinoma of the breast. Genetic and Molecular Research 2015;14(2):5417- 5426.
6. Hu R, Dawood S, Holmes MD, Collins LC, Schnitt SJ, Cole K et al. Androgen receptor expression and breast cancer survival in postmenopausal women. Clinical Cancer Research 2011;17(7):1867-1874.
7. Nigam JS and Sood N,. Correlation of CK5 and EGFR with clinicopathological profile of triple-negative breast cancer. Pathology Research International 2014;2014:1-6.
8. Jana D, Sarkar DK, Ganguly S, Banerjee A, Manna AK, Mandal S. Can molecular subtyping replace axillary nodal status as prognostic marker in breast cancer?. Indian Journal of Surgical Oncology 2014;5(4):282-289.
9. Akhtar M, Dasgupta S, Rangwala M. Triple negative breast cancer: an Indian perspective. Breast Cancer: Targets and Therapy 2015;7:239-243.
10. Doval DC, Sharma A, Sinha R, Kumar K, Dewan AK, Chaturvedi H, et al. Immunohistochemical profile of breast cancer patients at a tertiary care hospital in New Delhi, India. Asian Pacific Journal of Cancer Prevention 2015;16(12):4959-4964.
11. Patnayak R, Jena A, Rukmangadha N, Chowhan AK, Sambasivaiah K, Phaneendra BV, et al. Hormone receptor status (estrogen receptor, progesterone receptor), human epidermal growth factor-2 and p53 in South Indian breast cancer patients: A tertiary care center experience. Indian Journal of Medical and Paediatric Oncology: Official Journal of Indian Society of Medical & Paediatric Oncology 2015;36(2):117-122..
12. 12.Boyle P, Holmes S, William K. Triple-negative breast cancer: epidemiological considerations and recommendations. Annals of Oncology 2012;23(7):7-12.
13. Rao C, Shetty J, Prasad HK. Morphological profile and receptor status in breast carcinoma: An institutional study. Journal of Cancer Research and Therapeutics 2013;9(1):44-49.
14. Sen S, Gayen R, Das S, Maitra S, Jha A, Mahata M. A clinical and pathological study of triple negative breast carcinoma: experience of a tertiary care centre in eastern India. Journal of the Indian Medical Association 2012;110(10):686-689.
15. Singh R, Gupta S, Pawar SB, Pawar RS, Gandham SV, Prabhudesai S. Evaluation of ER, PR and HER-2 receptor expression in breast cancer patients presenting to a semi urban cancer centre in Western India. Journal of Cancer Research and Therapeutics 2014;10(1):26-28.
16. Dent R, Hanna WM, Trudeau M, Rawlinson E, Sun P, Narod SA. Pattern of metastatic spread in triple-negative breast cancer. Breast Cancer Research and Treatment 2009;115(2):423-428.
17. Suresh P, Batra U, Doval DC. Epidemiological and clinical profile of triple negative breast cancer at a cancer hospital in North India. Indian Journal of Medical and Paediatric Oncology: Official Journal of Indian Society of Medical& Paediatric Oncology. 2013;34(2):89-95.

18. Bashir WS, Ahmad SS, Mansoor L, Ahmad SM, Manzoor NA, Ashraf TM, et al. Incidence and clinical profile of Triple negative breast cancer in Kashmir. Journal of Medical Science and Clinical research 2017;5(4):20746-20749.
19. Arora A, Chaturvedi R, Goel V, Bohara S, Gupta V, Shukla M, et al. Expression of Androgen Receptor in Triple Negative Breast Cancer Biopsies and Its Correlation with Prognostic Markers. ***Indian Journal of Pathology: Research and Practice*** 2019;8(2):144-152.
20. Nabi M, Ahangar A, Wahid A, Kuchay S. Clinicopathological comparison of triple negative breast cancers with non-triple negative breast cancers in a hospital in North India. Nigerian J Clin Prac. 2015;18(3):381-86.
21. Mane A, Khatib KI, Deshmukh SP, Nag SM, Sane SP, Zade BP. A comparison of clinical features, pathology and outcomes in various subtypes of breast cancer in Indian women. Journal of Clinical and Diagnostic Research 2015;9(9):1-4.
22. Pareja F, Geyer FC, Marchiò C, Burke A, Weigelt B,Filho S. Triple-negative breast cancer: the importance of molecular and histologic subtyping, and recognition of low-grade variants. NPJ Breast Cancer 2016;16(2):166-172.
23. Kim J, Eom K, Koo R, Kim H, Kang E. A prognostic model for patients with triple-negative breast cancer: importance of the modified nottingham prognostic index and age. Journal of Breast Cancer 2017;20(1):65-73.
24. Urru SA, Gallus S, Bosetti C, Moi T, Medda R, Sollai E, et al. Clinical and pathological factors influencing survival in a large cohort of triple-negative breast cancer patients. BMC Cancer 2018;18(1):56-66.
25. Reddy M, Barcenas H, Sinha A, Hsu L, Moulder L, Tripathy D. Long-term survival outcomes of triple-receptor negative breast cancer survivors who are disease free at 5 years and relationship with low hormone receptor positivity. British Journal of Cancer 2018;118(1):17-23.
26. Agarwal C, Pujani M, Misra S, Srivastava A, Singh S. Circulating tumor cells in breast cancer: correlation with clinicopathological parameters, hormone profile and MicroRNA polymorphisms. *Turkish Journal of Pathology*2015;32(3):148-157.
27. Astvatsaturyan K, Yue Y, Walts AE, Bose S. Androgen receptor positive triple negative breast cancer: Clinicopathologic, prognostic, and predictive features. PloS one 2018;13(6):1-10.
28. Liu YX, Zhang KJ, Tang LL. Clinical significance of androgen receptor expression in triple negative breast cancer-an immunohistochemistry study. Oncology Letters 2018;15(6):10008-10016.
29. McGhan LJ, McCullough AE, Protheroe CA, Dueck AC, Lee JJ, Nunez-Nateras R, et al. Androgen receptor-positive triple negative breast cancer: a unique breast cancer subtype. Annals of Surgical Oncology 2014;21(2):361-367.
30. Teoh PY, Tan GC, Mahsin H, Wong YP. Androgen receptor expression in triple negative breast carcinoma and its association with the clinicopathological parameters. The Malaysian Journal of Pathology 2019;41(2):125-132.
31. Tan MH, Li J, Xu HE, Melcher K, Yong EL. Androgen receptor: structure, role in prostate cancer and drug discovery. ***Acta*** Pharmacologica ***Sinica*** 2015;36:3-23.
32. Sunar V, Dogan HT, Sarici F, Ates O, Akin S, Baspinar B, et al. Association between androgen receptor status and prognosis in triple negative breast cancer. Journal of BUON.: Official Journal of the Balkan Union of Oncology 2018;23(5):1325-1330.
33. Shin E, Jung WH, Koo JS. Expression of p16 and pRB in invasive breast cancer. International Journal of Clinical and Experimental Pathology 2015;8(7):8209-8217.

34. Bogina GS, Lunardi G, Marcolini L, Brunelli M, Bortesi L, Marconi M, et al. P16 but not retinoblastoma expression is related to clinical outcome in no-special-type triple-negative breast carcinomas. Modern Pathology 2014;27(2):204-213.
35. Abou-Bakr AA and Eldweny HI. p16 expression correlates with basal-like triple-negative breast carcinoma. Ecancermedicalscience 2013;7:317-328.
36. Hashmi AA, Naz S, Hashmi SK, Hussain ZF, Irfan M, Khan EY, et al. Prognostic significance of p16 & p53 immunohistochemical expression in triple negative breast cancer. BMC Clinical Pathology 2018;18(1):9-19.

Table 1. Clinicopathological features of 94 TNBC cases.

Variables	Number of TNBC cases	Percentage (%)
Specimen		
Mastectomy	88	93.6 %
Lumpectomy	6	6.4 %
Menopausal status		
Premenopausal	57	60.6 %
Postmenopausal	37	39.4 %
Age group (in years)		
20-29	8	8.5 %
30-39	20	21.3 %
40-49	32	34 %
50-59	22	23.4 %
≥ 60	12	12.8 %
Breast laterality		
Right	46	48.9 %
Left	48	51.1 %
Site involved		
Upper outer quadrant	56	59.6 %
Upper inner quadrant	12	12.8 %
Lower outer quadrant	10	10.6 %
Lower inner quadrant	4	4.2 %
Central	6	6.4 %
More than one quadrant	6	6.4 %
Clinical Presentation		
Breast lump	80	85.1 %
Nipple discharge	2	2.1 %

Ulceration	12	12.8 %
Tumor size		
≤ 2 cm	5	5.3 %
>2 - ≤5 cm	68	72.3 %
>5 cm	21	22.4 %
Histomorphological type		
Invasive carcinoma (NST)	92	97.8 %
Invasive lobular carcinoma	1	1.1 %
Carcinoma with Medullary features	1	1.1 %
Grade * (MBR – Modified Bloom Richardson Grade)		
Grade 1	3	3.2 %
Grade 2	30 (29 +1)**	32.3 %
Grade 3	60	64.5
Lymphovascular invasion		
Yes	23	24.5 %
No	71	75.5 %
Lymph Node(s) involvement (67 cases received with LN(s))		
Yes	42	62.7 %
No	25	37.3 %
No. of LN (s) involved (Out of 42 LN positive cases)		
1-3	30	71.4 %
4-9	10	23.8 %
≥ 10	2	4.8 %
Stages (For 67 cases received with LN(s))		
IA	3	4.5 %
IB	0	0 %
II A	24	35.8 %
II B	27	40.3 %
III A	8	11.9 %
III B	3	4.5 %
III C	2	3 %

***1 case of Carcinoma with medullary features - always considered as high grade. ** 29 Invasive Ca. (NST) + 1 Invasive Lobular Ca.**

Table 2. Clinicopathological features of AR positive and AR negative TNBC.

Variables	No. of cases	AR Expression		p – value****
		AR +	AR -	
TNBC cases	94	38 (40.4%)	56 (59.6%)	NA
Menopausal status				
Premenopausal	57	22 (38.6%)	35 (61.4%)	0.6537
Postmenopausal	37	16 (43.2%)	21 (56.8%)	
Age group (in years)				
20-29	8	2 (25%)	6 (75%)	0.1742
30-39	20	6 (30%)	14 (70%)	
40-49	32	15 (46.9%)	17 (53.1%)	
50-59	22	7 (31.8%)	15 (68.2%)	
≥ 60	12	8 (66.7%)	4 (33.3%)	
Tumor size				
≤ 2 cm	5	1 (20%)	4 (80%)	**0.0174**
>2 - ≤5 cm	68	23 (33.8%)	45 (66.2%)	
>5 cm	21	14 (66.7%)	7 (33.3%)	
Histomorphological type				
Invasive carcinoma (NST)	92	38 (41.3%)	54 (58.7%)	NA
Invasive lobular carcinoma	1	0 (0%)	1 (100%)	
Carcinoma with medullary features	1	0 (0%)	1 (100%)	
Grade* (MBR – Modified Bloom Richardson)				
Grade 1	3	2 (66.7%)	1 (33.3%)	0.5882
Grade 2	30 (29 +1)**	13 (43.3%)	17 (56.7%)	
Grade 3	60	23 (38.3%)	37 (61.7%)	
Primary Tumor Stage (T)				

T1	5	1 (20%)	4 (80%)	0.0591
T2	61	20 (32.8%)	41 (67.2%)	
T3	16	9 (56.3%)	7 (43.7%)	
T4	12	8 (66.7%)	4 (33.3%)	
Lymphovascular invasion				
Yes	23	15 (65.2%)	8 (34.8%)	**0.0053**
No	71	23 (32.4%)	48 (67.6%)	
Lymph Node(s) involvement (67 cases received with LN (s))* **				
Yes	42	22 (52.4%)	20 (47.6%)	**0.0227**
No	25	6 (24%)	19 (76%)	
Lymph Node Stage (N) (For 67 cases received with LN(s))				NO/N1=0.539
N0	25	8 (32%)	17 (68%)	N0/N2=0.126
N1	30	12 (40%)	18 (60%)	N0/N3=0.128 N1/N2=0.270
N2	10	6 (60%)	4 (40%)	N1/N3=0.183
N3	2	2 (100%)	0 (0%)	N2/N3=0.515
Stages (For 67 cases received with LN(s))				
I	3	1 (33.3%)	2 (66.7%)	I/II/III=0.2720
II A	24	8 (33.3%)	16 (66.7%)	
II B	27	11 (40.7%)	16 (59.3%)	
III A	8	4 (50%)	4 (50%)	
III B	3	2 (66.7%)	1 (33.3%)	
III C	2	2 (100%)	0 (0%)	

***1 case of Carcinoma with medullary features - always considered as high grade. This case was AR negative.**

**** 29 Invasive Carcinoma (NST) + 1 Invasive Lobular Carcinoma case.**

***** Out of 94 TNBC cases, 67 cases received with LN. Out of the rest 27 TNBC cases, 10 were AR positive.**

****** Appropriate statistical test to calculate p value applied wherever applicable.**

Table 3. Clinicopathological features of p16 positive and p16 negative TNBC.

Variables	N0. Of TNBC cases	P16 Expression		p – value****
		P16 +	P16 -	
TNBC cases	94	68 (72.3%)	26 (27.7%)	NA
Menopausal status				
Premenopausal	57	45 (78.9%)	12 (21.1%)	0.0755
Postmenopausal	37	23 (62.1%)	14 (37.9%)	
Age group (in years)				
20-29	8	5 (62.5%)	3 (37.5%)	0.1298
30-39	20	16 (80%)	4 (20%)	
40-49	32	27 (84.4%)	5 (15.6%)	
50-59	22	14 (63.3%)	8 (36.4%)	
≥ 60	12	6 (50%)	6 (50%)	
Tumor size				
≤ 2 cm	5	4 (80%)	1 (20%)	0.7662
>2 - ≤5 cm	68	50 (73.5%)	18 (26.5%)	
>5 cm	21	14 (66.7%)	7 (33.3%)	
Histomorphological type				
Invasive carcinoma (NST)	92	68 (73.9%)	24 (26.1%)	NA
Invasive lobular carcinoma	1	0 (0%)	1 (100%)	
Carcinoma with medullary features	1	0 (0%)	1 (100%)	
Grade* (MBR – Modified Bloom Richardson)				
Grade 1	3	2 (66.7%)	1 (33.3%)	

Grade 2	30 (29 +1)**	27 (90%)	3 (10%)	**0.040**
Grade 3	60	39 (65%)	21 (35%)	
Primary Tumor Stage (T)				
T1	5	4 (80%)	1 (20%)	
T2	61	48 (78.7%)	13 (21.3%)	0.06814
T3	16	11 (68.8%)	5 (31.2%)	
T4	12	5 (41.7%)	7 (58.3%)	
Lymphovascular invasion				
Yes	23	13 (56.5%)	10 (43.5%)	0.0510
No	71	55 (77.5%)	16 (22.5%)	
Lymph Node(s) involvement (67 cases received with LN(s)) ***				
Yes	42	29 (69%)	13 (31%)	0.5414
No	25	19 (76%)	6 (24%)	
Lymph Node Stage (N) (For 67 cases received with LN(s))				
N0	25	19 (76%)	6 (24%)	
N1	30	22 (73.3%)	8 (26.7%)	0.7045
N2	10	6 (60%)	4 (40%)	
N3	2	1 (50%)	1 (50%)	
Stages (For 67 cases received with LN(s))				
I	3	2 (66.7%)	1 (33.3%)	
II A	24	19 (79.2%)	5 (20.8%)	

II B	27	20 (74%)	7 (26%)	I/II/III = 0.2660
III A	8	5 (62.5%)	3 (37.5%)	
III B	3	1 (33.3%)	2 (66.7%)	
III C	2	1 (50%)	1 (50%)	

***1 case of Carcinoma with medullary features - always considered as high grade. This case was p16 negative.**
**** 29 Invasive Carcinoma (NST) + 1 Invasive Lobular Carcinoma case.**
*****Out of 94 TNBC cases, 67 cases received with LN. Out of the rest 27 TNBC cases, 20 were p16 positive.**
****** Appropriate statistical test to calculate p value applied wherever applicable.**

Table 4. AR Expression Scoring Pattern of TNBC cases.

		AR Expression Scoring Pattern			
Variables	**No. of TNBC cases**	**0**	**1+**	**2+**	**p – value*****
Grade(MBR)					
Grade 1	3	1 (33.3%)	1 (33.3%)	1 (33.3%)	0.890
Grade 2	30	17 (56.7%)	7 (23.3%)	6 (20%)	
Grade 3	60	37 (61.7%)	13 (21.7%)	10 (16.6%)	
Total	**93***	**55**	**21**	**17**	
Primary Tumor (T)					T1/T2 = 1 T1/T3 = 1
T1	5	4 (80%)	0 (0%)	1 (20%)	T1/T4 = 0.33
T2	61	41 (67.2%)	10 (16.4%)	10 (16.4%)	T2/T3 = 0.78 T2/T4 = 0.22
T3	16	7 (43.6%)	5 (31.3%)	4 (25.1%)	T3/T4 = 0.40
T4	12	4 (50%)	6 (50%)	2 (16.7%)	
Total	**94**	**56**	**21**	**17**	
LN Stage (N)					N0/N1 = 0.852
N0	25	17 (68%)	5 (20%)	3 (12%)	

N1	30	18 (60%)	7 (23.3%)	5 (16.7%)	N0/N2 = 0.639 N0/N3 = 0.746 N1/N2 = 0.737 N1/N3 = 0.825 N2/N3 = 1
N2	10	4 (40%)	3 (30%)	3 (30%)	
N3	2	0 (0%)	1 (50%)	1 (50%)	
Total	**67****	**39**	**17**	**11**	

***1 case was of carcinoma with medullary features with AR expression score 0.**

****Out of 94 TNBC cases, 67 cases are received with lymph nodes.**

*****Appropriate statistical test to calculate p value applied wherever applicable.**

Table 5. P16 Expression Scoring Pattern of TNBC cases.

		P16 Expression Scoring Pattern				
Variables	**No. of TNBC cases**	**0**	**1-4**	**5-8**	**9-12**	**P value*******
		(Negative)	**(Weakly Positive)**	**(Moderately Positive**	**(Strongly Positive)**	
Grade*(MBR)						
Grade 1	3	1 (33.3%)	0 (0%)	1 (33.3%)	1 (33.3%)	0.174**
Grade 2	30	3 (10%)	6 (20%)	8 (26.7%)	13 (43.3%)	
Grade 3	60	21 (35%)	13 (20%)	12 (21.7%)	14 (23.3%)	
Total	**93***	**25**	**19**	**21**	**28**	
Primary Tumor (T)						T1/T2 = 0.966 T1/T3 = 0.711 T1/T4 = 0.538 T2/T3 = 0.487 T2/T4 = 0.197 T3/T4 = 0.688
T1	5	1 (20%)	1 (20%)	1 (20%)	2 (40%)	
T2	61	13 (21.3%)	11 (18%)	15 (24.6%)	22 (36.1%)	
T3	16	5 (31.3%)	4 (25%)	4 (25%)	3 (18.7%)	
T4	12	7 (58.4%)	3 (25%)	1 (8.3%)	1 (8.3%)	
Total	**94**	**26**	**19**	**21**	**28**	

LN Stage (N)						
N0	25	6 (24%)	4 (16%)	6 (24%)	9 (36%)	0.569****
N1	30	8 (26.7%)	6 (20%)	8 (26.7%)	8 (26.7%)	
N2	10	4 (40%)	3 (30%)	2 (20%)	1 (10%)	
N3	2	1 (50%)	1 (50%)	0 (0%)	0 (0%)	
Total	**67****	**19**	**14**	**16**	**18**	

***1 case was of carcinoma with medullary features with P16 expression score 0.**

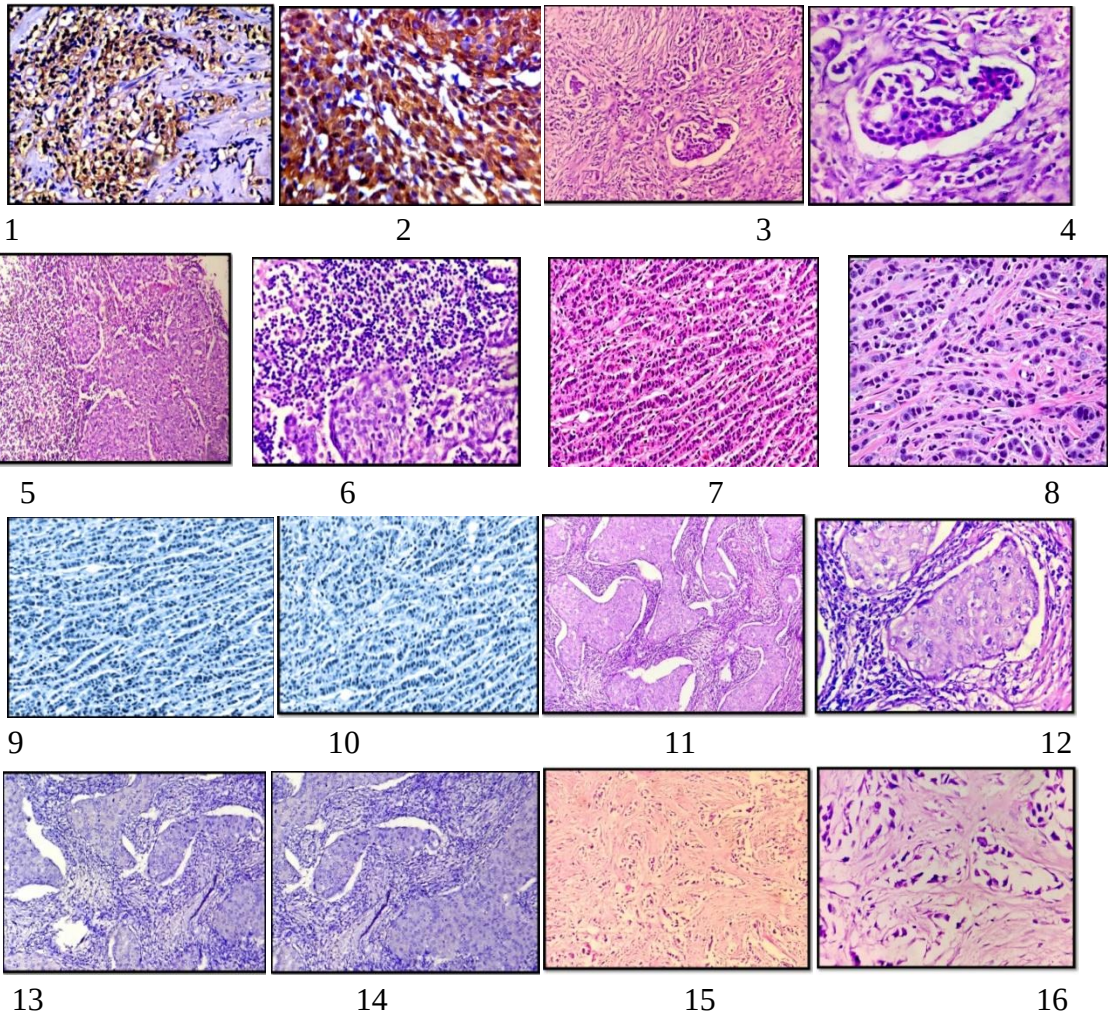
**** p value obtained among score 0-4,5-8 and 9-12.**

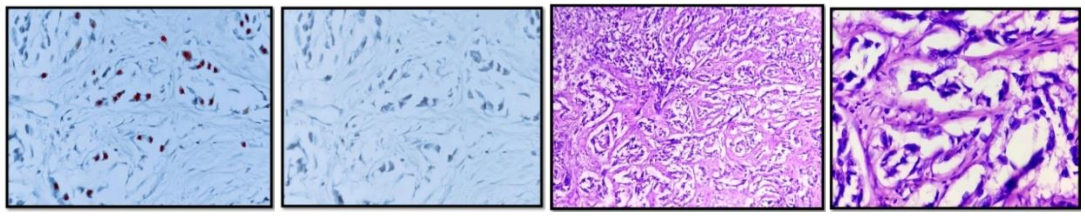
*****Out of 94 TNBC cases, 67 cases are received with lymph nodes.**

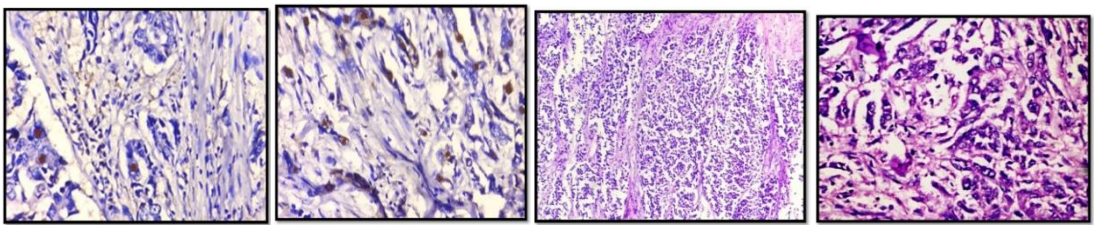
****** p value obtained among score N0,N1 and N2/N3 .**

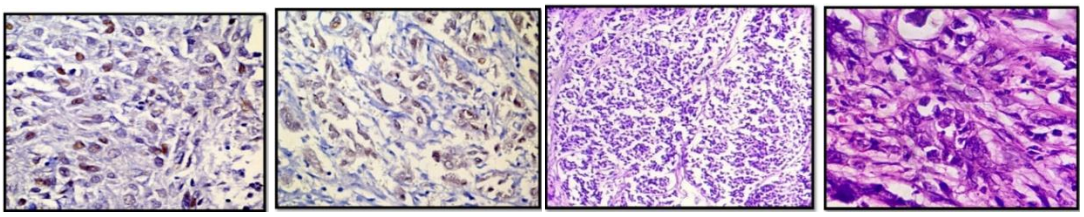
****** Appropriate statistical test to calculate p value applied wherever applicable.**

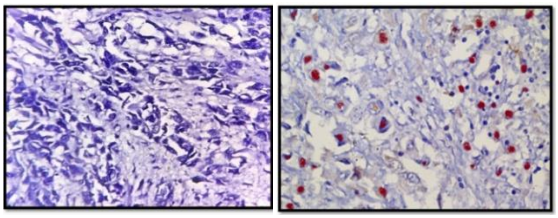
Histo morphological Images




17 18 19 20


21 22 23 24


25 26 27 28


29 30

1. HC (100x)-Positive control of androgen receptors expression in tumor cells of adenocarcinoma of prostate, showing nuclear positivity.
2. HC (400x) - Positive control of p16 expression in tumor cells of cervical carcinoma, showing nuclear as well as cytoplasmic positivity.
3. H & E (100x) - Showing vascular invasion of tumor cells in TNBC case.
4. H & E (400x)- Showing vascular invasion of tumor cells in TNBC case.
5. H & E (100x) - Showing lymph node metastases in TNBC case.
6. H & E (400x) - Showing lymph node metastases in TNBC case.
7. H & E (100x) – Showing TNBC with invasive lobular carcinoma histomorphology.
8. H & E (400x) – Showing TNBC with invasive lobular carcinoma histomorphology.
9. HC (100x) – Showing negative androgen receptor expression (score 0) in TNBC with invasive lobular carcinoma histomorphology.
10. IHC (100x) – Showing negative p16 expression (score 0) in TNBC with invasive lobular carcinoma histomorphology.
11. H & E (100x) – Showing TNBC with carcinoma with medullary features histomorphology.
12. H & E (400x) – Showing TNBC with carcinoma with medullary features histomorphology.
13. IHC (100x) – Showing negative androgen receptor expression (score 0) in TNBC with carcinoma with medullary features histomorphology.

14. IHC (100x) – Showing negative p16 expression (score 0) in TNBC with carcinoma with medullary features histomorphology.
15. H & E (100x) – Showing TNBC with invasive carcinoma (NST) histomorphology, grade 1
16. H & E (400x) – Showing TNBC with invasive carcinoma (NST) histomorphology, grade 1.
17. IHC (400x) – Showing androgen receptor nuclear positivity (Score 2+) in TNBC with invasive carcinoma (NST) histomorphology, grade 1.
18. IHC (400x) – Showing negative p16 expression (Score 0) in TNBC with invasive carcinoma (NST) histomorphology, grade 1.
19. H & E (100x) – Showing TNBC with invasive carcinoma (NST) histomorphology, grade 2.
20. H & E (400x) – Showing TNBC with invasive carcinoma (NST) histomorphology, grade 2.
21. HC (400x) – Showing androgen receptor nuclear positivity (Score 1+) in TNBC with invasive carcinoma (NST) histomorphology, grade 2.
22. IHC (400x) – Showing p16 nuclear as well as cytoplasmic positivity (Score 4+) in TNBC with invasive carcinoma (NST) histomorphology, grade 2.
23. H & E (100x) – Showing TNBC with invasive carcinoma (NST) histomorphology, grade 3.
24. H & E (400x) – Showing TNBC with invasive carcinoma (NST) histomorphology, grade 3.
25. IHC (400x) – Showing androgen receptor nuclear positivity (Score 2+) in TNBC with invasive carcinoma (NST) histomorphology, grade 3.
26. IHC (400x) – Showing p16 nuclear as well as cytoplasmic positivity (Score 8+) in TNBC with invasive carcinoma (NST) histomorphology, grade 3.
27. H & E (100x) – Showing TNBC with invasive carcinoma (NST) histomorphology, grade 3.
28. H & E (400x) – Showing TNBC with invasive carcinoma (NST) histomorphology, grade 3.
29. IHC (400x) – Negative androgen receptor expression (Score 0) in TNBC with invasive carcinoma (NST) histomorphology, grade 3.
30. IHC (400x) – Positive p16 nuclear as well as cytoplasmic expression (Score 9+) in TNBC with invasive carcinoma (NST) histomorphology, grade 3.