ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, S Iss 3, Dec 2022

TRIPLE NEGATIVE BREAST CANCER: A CLINICOPATHOLOGICAL STUDY

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ABSTRACT

"Cancer remains an affliction for mankind. After lung cancer, breast cancer happens to be the second most frequent form of cancer. Worldwide, it is the commonest cancer among females. It accounts for 11.6 % of all cancers with 2,088,849 new diagnosed cases and 626,679 yearly deaths in spite of refinements in diagnosis and treatment modalities".[1]

"Triple negative breast cancers are an intricate and composite group of cancers and have several molecular subtypes. Tumors that don't express estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor" [2] (HER2) "are called as triple negative breast cancer (TNBC).[2] The consequences in terms of survival and disease-free interval are poor.[3,4] They have a distinctive and peculiar pathological and molecular behavior".

INTRODUCTION

"TNBCs have multitude of unique hostile clinicopathological features, including young age of presentation and large tumor size".[9,10] "The histological findings include high grade, high proliferative activity, infiltrative margins, foci of necrosis, absence of gland formation, fibrotic foci and predominant lymphoplasmacytic infiltrates".[9,11,12,13] "TNBCs express proteins that are distinctive feature of basal epithelial cells of the breast which are associated with abrupt speedy multiplication and poor prognosis".[14]

"The primary option in TNBC management is Breast conservation treatment which is an effort to circumvent mastectomy. However, more and more tumor recurrences irrespective of radiation therapy calls the need for mastectomy adjuncted with radiotherapy.[15] TNBCs have no hormonal receptors which render hormonal therapy useless thus letting chemotherapy be the cornerstone of therapy".[16]

Objectives of This Study:

(i) To study the clinical profile of Triple Negative Breast Cancer.
(ii) To study and analyse different histomorphological types of Triple Negative Breast Cancer.

Review of Literature

The female breast has always been a part of beauty, femininity and fertility. Cancers and their existence have been traced back to the pre-historic times and mummification era. The Edwin Smith Surgical papyrus (3000-2500 BC) describes breast cancer as a bulging tumor. Hippocrates' classic descriptions of progressive stages of breast cancer represent early hypotheses on cancer.[121]

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Galen, in 200 AD, attributed breast cancer to be a systematic disease on account of black bile accumulation in blood. The word 'Cancer' was coined from the word Crab by him based on the dilated veins radiating from tumor. The use of caustic paste to eliminate the tumor and render it operable was developed between 476- 1500 AD which is the same concept used for chemotherapy for severe breast cancers nowadays.[121]

"Margottini and Veronesis in Milan also removed internal mammary lymph nodes, preserving Halstead's legacy. Patey and Handley from London and Auchincloss of New York ushered in the movement that 'modified' the radical mastectomy by preserving the pectoralis major muscle. Early detection of smaller lesions by mammography has lately added to a newer dimension to surgical management of breast cancer".[121]

"Estrogen is the main female hormone responsible for breast development and maintenance. It leads to growth of the ductal system and also maturation and prominence of the nipples, resulting in proliferation of the ductal epithelium, myoepithelial cells and surrounding stroma. Estrogen is lipid soluble and in a woman's body is made by the ovaries and to a lesser extent by the adrenal glands. It is stimulated to act in the presence of other hormones such as hydrocortisone, insulin-like growth factors and growth hormones."

"Progesterone is released by the ovaries and induces development of the terminal ducts and lobulo-alveolar structures. Like Estrogen, it needs the presence of the other hormones, such as growth hormones and insulin, to respond. Both Estrogen and progesterone can increase connective tissue and fat in the breast, thereby leading to the rounded form of the fully developed breast."

"Oxytocin is a peptide hormone that is synthesized in the hypothalamus and released by the posterior pituitary gland (neurohypophysis). The act of nursing (the sucking reflex) stimulates its release".[107,110,113,116] "Oxytocin causes myoepithelial cells to contract, which squeezes milk out from the lobules into the lactiferous ducts. Human Placental Lactogen (hPL) is produced by the maternal placenta and serum levels continue to rise throughout pregnancy. It is related to breast growth and differentiation during pregnancy and reaches a peak during the final weeks of gestation, preparing the breast for milk production. Its serum levels decline rapidly after birth."

During pregnancy, the breast reaches its maximum development.[107,110,113,116,117] "High levels of Estrogen and progesterone are maintained by the corpus luteum initially and by the placenta during the latter phase. Estrogen stimulates the ducts and lobules to proliferate and the glandular epithelium replaces the fat tissue. In the second trimester, progesterone activates the secretory epithelium. During the last trimester the alveolar and ductal spaces are filled with colostrum and towards the end of pregnancy there is a rise in prolactin circulation and the production of milk, fat and proteins begins."

"During the first days after birth, the mother's breast does not produce real milk, but it produces colostrum, with a high immunoglobulin concentration that enhances the underdeveloped immune system of the infant. After 5–7 days the milk becomes filled with all the nutrients and antibodies necessary for growth. When breastfeeding stops, the milk is being produced for several months but in lesser amounts. The breast usually returns to its previous size."

Breast Cancer Overview

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"Hormone receptor analysis is now an established procedure in routine management of breast cancer but the cost of evaluation and non-affordability are key concerns in performing hormone receptor analysis in an Indian scenario. With increasing prevalence of locally advanced breast cancer (LABC) and aggressive tumors it is a good rationale to evaluate hormonal status of breast cancer in central India as there is paucity of hormone receptor data. Triple negative breast cancer (TNBC) is a recent concept and hot topic for research. It is also associated with aggressive tumors, seen in a younger age group, with shorter disease-free survival."

Clinical Features

- A lump or thickening in the breast that feels different than the surrounding tissue Changes in the size and/or shape as well as appearance of the breast
- Redness or pitting of skin (orange-skin)
- Changes on the breast skin surface (such as dimpling)
- Nipple inversion and/or nipple discharge
- Scaling, crusting, ulceration, pigmentation on the skin surface
- Pain in the breast Weight loss
- Lump in axillary tail

Sonomammography

Ultra sound examination of the breast is carried out by the usage of a linear probe array while the patient lies in a supine position to examine the medial parts of the breast and in contralateral posterior oblique position with raised arms for the lateral parts of the breast. The diagnosis made is scored from BIRADS (Breast Imaging Reporting and Data System) categories 0 to 6.

"The most common special types of breast cancer include: medullary carcinoma, metaplastic carcinoma, apocrine carcinoma, mucinous carcinoma, cribriform carcinoma, tubular carcinoma, neuroendocrine carcinoma, classic lobular carcinoma and pleomorphic lobular carcinoma".[65]

Invasive breast carcinoma - no specific type (IBC-NST)

"The histological subtype IBC-NST is the most common, constituting about 40% to 75% of all invasive breast carcinomas. Usually, it has a wide scope of morphological variation and clinical behavior.[65] Tumor cells are pleomorphic, with protruding nucleoli and numerous mitoses. Areas of necrosis and calcifications can be detected in more than half of the cases".[62,65]

Medullary carcinoma

"Special subtype of invasive breast carcinoma, responsible for approximately 5% of all cases, and associated with better clinical results and lower rates of involvement in axillary lymph nodes.[66]It usually affects patients between 30 and 40 years old and is often associated with mutations in the BRCA1 germline (Breast cancer gene 1). [65] Microscopically, it is a well-circumscribed carcinoma, composed of large and pleomorphic tumor cells, with a syncytial growth pattern, frequent mitotic figures and prominent lymphoplasmacytic infiltrate. Other commonly seen features include spindle cell metaplasia and giant tumor cells".[67,68]

Metaplastic carcinoma

"This histological subtype is characterized by the dominant component of metaplastic differentiation, representing approximately 1% of all cases and affecting women, mainly in post-menopause.[69] This group of tumors shows aggressive biological behavior and an often lymph node involvement.[70] Morphologically,

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it is a poorly differentiated heterogeneous tumor that contains ductal carcinoma cells mixed with other histological elements, such as squamous cells, spindle cells or other mesenchymal differentiation, such as chondroid cells, bone cells, and myoepithelial cells". [67,70]

"It constitutes about 1% to 4% of all cases, with prominent apocrine differentiation comprising at least 90% of tumor cells.[62] This subtype is generally of high histological grade, with poor prognosis and affects a wide age group, but it is more commonly seen in postmenopausal women.[71] Microscopically, tumor cells are large, with an abundant granular eosinophilic cytoplasm, positive for PAS (Periodic acid-reactive Schiff) staining and prominent nucleoli; in addition, bizarre tumor cells with multilobulated nuclei can also be observed".[67,72]

Mucinous carcinoma

"It is a special subtype of breast cancer, also known as colloid, gelatinous, mucous and mucoid carcinoma, responsible for 2% of all newly diagnosed cases.[66] This subtype has been associated with a favorable prognosis and often affects women over 60 years of age.[73] Morphologically, these tumors have abundant amounts of extracellular mucin, surrounding small clusters of tumor cells with different growth patterns and with mild nuclear atypia".[67,74]

Cribriform carcinoma

"Special subtype associated to a good prognosis, generally affecting patients who are approximately 50 years old and constituting about 1% to 3.5% of all breast cancer cases.[61]Cribriform carcinoma has almost no evidence of regional or distant metastasis".[62]

Microscopically, "this subtype presents islands of uniform tumor cells, with low- grade atypia, cribriform appearance in 90% of the tumor and often associated with DCIS (Ductal carcinoma in situ) without well-defined stromal invasion".[75]

Tubular carcinoma

"Well-differentiated subtype, occurring in women between 50 and 60 years of age and constituting about 2% of all newly diagnosed cases.[66] Most tubular carcinomas are associated to a wide range of potentially premalignant proliferative lesions."

Immunohistochemical Interpretation

"Immunohistochemical assessment of hormone receptors is performed by using two parameters, namely the number of positively stained tumour cell nuclei and the intensity of staining. Many scoring systems have been proposed for assessing both the parameters, of which Allred scoring system is the simpler and most widely accepted and recommended method. The number of stained tumour cell nuclei is expressed as percentage of total tumour cell population."

Molecular Classification of Breast Cancer

"Breast cancer represents a biologically and phenotypically heterogeneous collection of diseases with different clinical and treatment response behaviors.[81] Only the morphological classification (nuclear grade, tubular grade, mitotic index, histological grade, and architectural characteristics) and the clinical pathological parameters (tumor size, lymph node involvement, metastasis), are insufficient to predict the real behavior of breast tumor pathophysiology".[65,82]

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"Thus, many studies focus on analyzing the molecular patterns of breast cancer in order to group these tumors into classes or entities to assist in clinical management, in the preparation of epidemiological and functional studies and in the performance of clinical trials. The pioneering work by Perou, Sorlie and colleagues at the beginning of this millennium classified breast cancer molecularly into distinct subgroups, based on similarities in gene expression profiles, using the cDNA microarray technique".[86,88,89]

Materials and Methods

- **Study Design**:

This study is an observational and analytical, prospective (2 years) and retrospective (3 years) study.

- **Study Setting**:

This included all specimens of lumpectomy or modified radical mastectomy that were received in the department of surgical pathology, in Krishna Institute of Medical Sciences Hospital, Karad from June 2016 to May 2021.

- **Selection Of Cases**:

Data was collected over a period of 18 months.

- **Study Location**:

The study was performed in the Histopathology section of Department of Pathology, Krishna Institute of Medical Sciences, Karad.

- **Inclusion Criteria**:

All subjects undergoing surgically excised lumpectomy or modified radical mastectomy are included in this study. The cases diagnosed as TNBC were taken for detailed study.

- **Exclusion Criteria**:

Subjects with recurrent breast cancer and/or subjects undergoing chemotherapy were excluded from the study

- **SAMPLE SIZE:**

According to the study Thike, A., Cheok, P., Jara-Lazaro, A. *et al* Triple- negative breast cancer: clinicopathological characteristics and relationship with basal-like breast cancer. *Mod Pathol* 23**,** 123–133 (2010)[143]

$$\text{Using the formula, } n = \frac{4pq}{L^2}$$

Where n = Sample size, p = prevalence, q = 100-p, L = Allowable error (10%),the minimum cases to be selected for this study will be **100.** However, we have included **302** cases in this study.

- **ETHICAL CONCERN**: Ethical clearance has been obtained from the EthicalCommittee.
- **RISK FACTOR IN DETAILS**: There is no risk factor in association with thestudy.

Methodology:

Data Collection:

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The data including patients' age, clinical staging, and other clinical data were obtained from the pathology records.

Grossing Of Specimen:
Lumpectomy or modified radical mastectomy specimens received were grossed according to routine protocol wherein bits from tumor, margins, overlying skin, nipple and areola as well as lymph nodes were submitted. These were further fixed in 10% NBF or neutral buffered formalin and processed according to recommended routine guidelines.

10% neutral buffered formalin (NBF) that is used for fixation was prepared by the following constituents:

40% formalin - 100 ml Water - 900 ml

Sodium dihydrogen phosphate monohydrate - 4 grams

Anhydrous disodium hydrogen phosphate - 6.5 grams TECHNIQUE OF TISSUE

Processing:

(i) Tissue Dehydration – 3 changes of graded alcohol followed by 2 changes of acetone.
(ii) Clearing – by means of clearing agent Chloroform.
(iii) Paraffin impregnation – 2 changes at a temperature of 60ºC
(iv) Tissue embedding - in paraffin wax, labeled appropriately and blocks were made after trimming the excess paraffin on the sides.
(v) Section cutting - Sections were cut with the help of a rotary microtome on a setting of 4 microns.
(vi) The sections were further floated on a water bath having a 60ºC temperature set.
(vii) Section mounting was done on a slide using a very fine and thin layer of glycerol egg albumin as an adhesive.
(viii) For immunohistochemical study, the section mounting was done on slides coated with Poly-L-Lysine.

Staining Protocol and Procedure:
Sections of 3-4µm thickness were cut and stained with Haematoxylin & Eosin stain.

Procedure of H&E staining:
Step 1) Deparaffinization - Sections are de-paraffinised

Step 2) Rehydration - hydrated by means of graded alcohol to water.

Step 3) Nuclear Staining - Stained with alum hematoxylin for nearly 5-15 minutes.
Then the slides are washed under running tap water for 5 minutes.

Step 4) Differentiation – Slides are differentiated in 1% acid alcohol for about 5 seconds.
Then, they are washed well in tap water.

Step 5) Bluing – Sections are dipped to be blued by using an alkaline solution (ammonia water)

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followed by a tap water wash.

Step 6) Counterstaining – Sections are then counterstained with Eosin Y (1%) for approximately 15 seconds. Sections are then washed in running tap water for 1-5 minutes.

Step 7) Dehydration – by means of graded alcohol

Step 8) Clearing is done using Xylene and coverslip is mounted by using DPX. The slides were studied under light microscopy and the data recorded.

Results of H & E staining:
Nuclei – blue to black due to hematoxylin. Cytoplasm and other substances – pink due to eosin

Results and Observations
During the study period, 302 specimens of modified radical mastectomy or lumpectomy that fulfilled the inclusion/exclusion criteria were received in the department of histopathology.

The histological types of these specimens were diagnosed as Invasive Breast Carcinoma NST (250 out of 302 specimens, 82.7%), followed by Invasive Lobular Carcinoma, Mucinous Carcinoma, Medullary Carcinoma, Apocrine Carcinoma, Metaplastic Carcinoma, Neuroendocrine Carcinoma, Micropapillary Carcinoma, etc.

These 302 specimens were further subjected to Immunohistochemistry based on ER, PR and HER 2 Neu expression.

It was observed that 100 out of 302 (33.1%) cases were negative for all the three markers and were thus marked as TRIPLE NEGATIVE BREAST CANCERs.

These 100 cases were studied in detail based on clinicopathological parameters.

Discussion
Of the 302 specimens included in this study, the most common histological type was Invasive Breast Carcinoma NST (250 out of 302 specimens, 82.7%), followed by Invasive Lobular Carcinoma, Mucinous Carcinoma, Medullary Carcinoma, Apocrine Carcinoma, Metaplastic Carcinoma, Neuroendocrine Carcinoma, Micropapillary Carcinoma, etc.

Upon immunohistochemistry for ER, PR and Her 2 expression, it was found out that 100 of these 302 cases (33.1%) were negative for all three markers and thus labelled Triple Negative Breast Cancer or TNBC. This finding is concordant with Nabi et. al, 2015[133] (34.4%), Sharma et. al. 2014[134] (31.9%), Singh et. al., 2014[135] (34.1%) and Ghosh et. a., 2011[136] (31%). These 100 cases were studied in detail based on clinicopathological parameters.

Of the 100 patients, 40 patients (40%) had taken hormonal therapy, 56 patients had not taken hormonal therapy (56%) and data was unavailable in 4 cases (4%). The median duration of a palpable lump was 5 months. Twelve cases (12%) of the 100 studied showed a positive family history. FNAC was positive for carcinoma cells in 96 of the 100 cases. (96%)

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Sonomammography results showed: 18 cases in BIRADS IV A (low suspicion of malignancy) category (18%), 44 cases in BIRADS IV B (medium suspicion of malignancy) category (44%), 22 cases in BIRADS IV C (high suspicion of malignancy) category (22%), 14 cases in BIRADS V (suggestive of malignancy) category (14%). 2 cases had unavailable details (2%).

Tumor showed more laterality to the right breast in 60 cases of 100 (60%) than in the left breast (40%).

Summary and Conclusions

- A total of 302 cases were included in this study. The most common histological type was found to be Invasive Breast Carcinoma NST (82.7%).
- Immunohistochemistry revealed 100 (33.1%) cases to be TNBC, which were further studied in detail.
- Most common age group was perimenopausal age group (41-60 years) (60%) with mean age at 52.3 years.
- Most cases (58%) were menopausal. In our study, 40% patients had taken hormonal therapy. Mean duration of lump was 5 months.
- A 12% of cases showed positive family history, 96% cases had positive FNA findings and most cases were diagnosed with BIRADS IV B (44%) on sonomammography.
- Tumor showed laterality to right breast (60%) and most commonly the tumor size was between 2-5 cm (46%).

To improve therapeutic outcome of TNBC, reliable predictive biomarkers and newer drugs against the known molecular pathways are required. Oncopathologist plays a vital role in the diagnostic and prognostic aspect of TNBCs.

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