Section: Pathology

Original Research Article

EVALUATION OF ANAPLASTIC LYMPHOMA KINASE (ALK) IMMUNOHISTOCHEMICAL EXPRESSION IN PATIENTS WITH TRIPLE NEGATIVE BREAST CANCER

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ABSTRACT

Background: Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer-related deaths among women worldwide. Anaplastic lymphoma kinase is a gene belonging to the tyrosine kinase family, associated with various cancers, including triple negative breast cancer.

Materials and Methods: A total of 75 cases with triple negative breast cancer were included in this retrospective study. The study was conducted in the Department of Pathology, Malla Reddy Medical College for Women over a period of 2 years, i.e. from January 2022 to January 2024.

Results: The mean age of the study population was 53.6 years. Invasive ductal carcinoma was the most common diagnosis. 30.6% of the study population showed ALK expression.

Conclusion: Triple negative breast cancer is associated with poor prognosis. Determining the levels of ALK immunoexpression will aid in the initiation of treatment with ALK inhibitors in patients with triple negative breast cancer.

Keywords: triple negative breast cancer, anaplastic lymphoma kinase, immunoexpression.

INTRODUCTION

Breast cancer is a significant health concern worldwide, and its epidemiology varies across populations. Several factors contribute to the development of breast cancer, including age, family history, genetic mutations (e.g., BRCA1 and BRCA2), hormonal factors (e.g., early menarche, late menopause, hormone replacement therapy), lifestyle factors (e.g., obesity, alcohol consumption), and environmental factors.

Triple-negative breast cancer (TNBC) represents a subtype of breast cancer that lacks expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). It accounts for approximately 10-15% of all breast cancer cases and is more common in younger age group, women with African origin and women with BRCA1 mutations.

TNBC tends to be more aggressive, with higher rate of recurrence and poor prognosis compared to other breast cancer subtypes and has limited treatment options available due to absent hormone receptor expression.[1-4]

Anaplastic lymphoma kinase (ALK) is a gene that codes for a tyrosine kinase receptor. Genetic abnormalities such as gene fusions or mutations have been implicated in various cancers, like anaplastic large cell lymphoma (ALCL), non-small cell lung cancer (NSCLC) but are relatively rare in triple-negative breast cancer (less than 1-2% of cases).[5-6]

Animal studies on mice have demonstrated that inhibition of ALK receptor inhibits the growth of breast cancer cell lines and also the tumor xenografts. Studies have shown that ALK aberrations such as increased ALK copy number, gene amplification and translocation are present in 80 % of inflammatory breast cancer and 25 % of triple-negative breast cancers (TNBC), which are considered to be the most aggressive subtypes of breast cancer.[7,8]

Owing to the poor prognosis of TNBC and lack of hormonal therapy, identification of ALK aberrations and using them as a potential therapeutic target can be used in the management of triple negative breast cancers.

This study was conducted with the aim to evaluate ALK expression in TNBC and to assess the significance of the same.

MATERIALS AND METHODS

This retrospective study was conducted in the Department of Pathology, Malla Reddy Medical College for Women over a period of 2 years, i.e. from January 2022 to January 2024. This study was initiated after the ethical committee approval.

All patients with triple negative breast cancer (TNBC) who were operated upon in the institution were included in the study. Patients with insufficient tissue samples and who were operated outside the institution were excluded from the study. A total of 75 patients were included in the study.

The clinical (age, presentation, demographic details and examination findings) and histopathological (tumor size, histological type, grade, stage, IHC for ER, PR and HER2) data of the breast cancer cases were retrieved.

The tissue blocks were fixed using a 10% neutral buffered formalin solution and embedded with paraffin. Sections were generated from the tissue blocks for staining with routine Haematoxylin and Eosin (H&E) stain and ALK immunohistochemical staining (using anti-human CD246 ALK antibody). The tumors having cytoplasmic and/or nuclear expression of ALK in more than 10% of the tumor cells were considered positive whereas the tumors with expression in less than 10% of the tumor cells were considered negative for ALK expression. The histologic subtypes were classified according to the World Health Organization (WHO) classification. Grading of the tumors were done based on the Modified Bloom-Richardson scores for tubular differentiation, nuclear pleomorphism and mitotic rate into Grade 1 (for a total score of 3-5), Grade 2 (for total score of 6-7) and Grade 3 (for a total score 8-9).[9]

All the statistical data were analyzed using SPSS software version 20.0. For quantitative variables, mean and standard deviation were used and for qualitative variables, prevalence and ratio were used. Chi-square test, Fisher's exact test were used to detect significance, 2-sided significance for each were considered. All p-values of <0.05 resulting from two-sided tests were considered significant.

RESULTS

A total of 75 patients with triple negative breast cancer were included in the study. The study population was aged from 28 – 72 years with a mean age of 53.6 years.

Table 1: Age- wise distribution of triple negative breast cancers

Age in years	Number of cases	Percentage %
21-30 years	2	2.6%
31-40 years	14	18.6%
41-50 years	20	26.6%
51-60 years	30	40%
61-70 years	8	10.6%
>70 years	1	1.3%
Total	75	100

The size of the tumors ranged between 1cm and 10cm with a mean size of 4.1 cm. Invasive ductal carcinoma was the most common histological subtype, followed by invasive lobular carcinoma.

Table 2: Histological variants of triple negative breast cancers

Histological type	Number of cases	Percentage %
No special type	**48**	**64%**
Lobular	**9**	**12%**
Medullary	**7**	**9.3%**
Papillary	**6**	**8%**
Metaplastic	**2**	**2.6%**
Secretory	**2**	**2.6%**
Adenoid cystic carcinoma	**1**	**1.3%**
Total	**75**	**100**

Grading of the tumors were done according to the Modified Bloom-Richardson Score. The majority of the patients had Grade 2 (40%), followed by Grade 1 tumors (38.6%). The tumor status and nodal status according to TNM classification, classified majority of the cases as T2 (45.3%) and N1 (40.0%) stages.

Table 3: Staging and grading of triple negative breast cancers

GRADE		
• 1	29	38.6%
• 2	30	40%
• 3	16	21.33%
TUMOR STATUS		
• T1	12	16%
• T2	34	45.3%
• T3	19	25.3%
• T4	10	13.33%

NODAL STATUS		
• N0	10	13.33%
• N1	30	40.0%
• N2	23	30.6%
• N3	12	16%

Amongst the 75 cases with TNBC, 23 (30.6%) showed positive immunoexpression for ALK, which was diffuse cytoplasmic positivity predominantly. However, a few nuclei also showed positive expression.

Table 4: ALK distribution in histological subtypes of TNBC

HISTOLOGICAL TYPE	NUMBER OF CASES	ALK POSITIVE CASES	ALK NEGATIVE CASES	p VALUE
No special type	48	12	36	0.0089
Lobular	9	5	4	0.7145
Medullary	7	2	5	0.0748
Papillary	6	2	4	0.2074
Metaplastic	2	1	1	0.4825
Secretory	2	1	1	0.2952
Adenoid cystic carcinoma	1	0	1	1.00
Total	75	23	52	

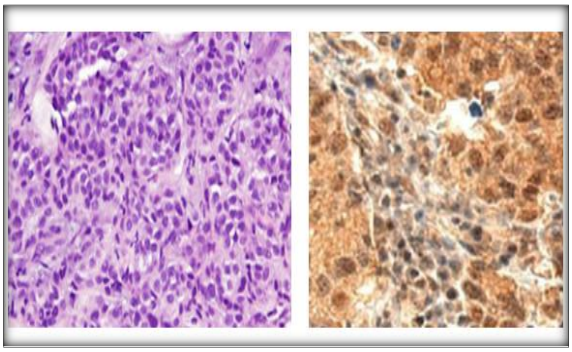

Figure 1: H & E staining (Figure 1) and ALK immunohistochemical staining of inflammatory breast cancer (Figure 2)

DISCUSSIONS

Anaplastic lymphoma kinase (ALK) aberrations, such as gene fusions or mutations, are relatively rare in triple-negative breast cancers (TNBC). However, when present, they may have important clinical implications. The presence of hormone receptors in tumors makes them amenable to hormonal therapy. It is always the tumors with negative hormone receptor status that poses a real challenge in cancer therapy.

In this retrospective study, a total of 75 triple negative breast cancer patients were included to evaluate the immunoexpression of anaplastic lymphoma kinase (ALK).

In the present study, 30.6% of the patients had ALK immunoexpression. Study done by Bassam et al,[10] also had a similar positivity rate (29.5%), whereas in study done by Perez-Pinera P et al,[11] all the cases showed ALK expression.

This could be attributed to the difference in geographical distribution of the study population in these studies.

Similar to the present study where majority of the cells had cytoplasmic ALK positivity, studies done by Bassam et al,[10] and Pinera et al,[11] also showed similar ALK expression. This can be explained by ALK receptors being localized to the cytoplasm and it is the site of aberrations.

Limitations of the study

The main limitation of the present study is a small sample size. Prospective studies involving larger groups from different geographical and ethnic groups with more specific methods of ALK detection are required to reach a definite conclusion

CONCLUSION

Triple negative breast cancer is usually associated with poor prognosis due to absence of hormone receptor expression, which make them resistant to treatment with hormonal therapy. Detection of ALK expression in them makes them amenable to treatment with ALK inhibitors such as Crizotinib.

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Conflicts of Interest: NIL.

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