E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.48047/ijprt/15.02.330

Research Article

IMMUNOHISTOCHEMICAL EXPRESSION OF MYELOID CELL LEUKEMIA-1 AND ITS CORRELATION WITH ER, PR, HER 2-NEU +/- CASES OF CARCINOMA BREAST

Dr. Shilpi Patel[1], Dr. Nishi Tandon[2], Dr. Andleeb Zehra[3*], Dr. Farheen Khan[4], Dr. Sharique Ahmad[5], Dr.NirupmaLal[6]

[1]MBBS, Postgraduate Junior Resident, Department of Pathology Era Lucknow Medical College and hospital, Lucknow. Orcid id –https://orcid.org/0009-0009-3797-0143
[2]MD Pathology, Professor, Department of Pathology, Era Lucknow Medical College and Hospital, Lucknow. Orcid id –https://orcid.org/0000-0002-3710-2743
[3]MD Pathology, Assistant Professor, Department of Pathology, Era Lucknow Medical College and Hospital, Lucknow. Orcid id - https://orcid.org/0000-0002-7547-0360
[4]MBBS, Postgraduate Junior Resident, Department of Pathology, Era Lucknow Medical College and Hospital, Lucknow.
[5]MD Pathology, Professor, Department of Pathology, Era Lucknow Medical College and Hospital, Lucknow. Orcid id - https://orcid.org/0000-0002-9637-8838
[6]MD Pathology, Professor and Head, Department of Pathology, Era Lucknow Medical College and Hospital, Lucknow. Orcid id - https://orcid.org/0000-0001-9615-5426

Corresponding Author: Dr. Andleeb Zehra
MD Pathology, Assistant Professor, Department of Pathology, Era Lucknow Medical College and Hospital, Lucknow. Orcid id - https://orcid.org/0000-0002-7547-0360

Received date: 10-10-2025, Date of acceptance: 15-10-2025, Date of publication: 15-10-2025

ABSTRACT

Background & Objectives: Breast carcinoma, the foremost typical cancer and fatal cause among women worldwide, is marked by unrestrained growth of breast epithelial cells.It's prognosis depends on biomarkers including Progesterone Receptor (PR), HER2-neu and Estrogen Receptor (ER). Myeloid cell leukemia-1 (MCL-1), an apoptosis inhibitor indicating poor survival, has gained attention as a promising therapeutic target, especially in triple-negative subtype of breast carcinoma (TNBC).It has been proposed that MCL-1 expression may be induced prior to BCL-2 expression in certain cell types, especially under stress conditions. This early induction of MCL-1 provides a rapid, albeit short-term, protection against apoptosis. Over time, the shift towards increased BCL-2 expression allows for sustained survival signals, maintaining the balance between cell death and survival pathways [22] The study intends to explore clinical importance and molecular mechanisms of MCL-1,highlighting correlations with HER2-neu, PR, ER, and its potential as a drug target.

(ii)Methods: Study analyzed histopathological samples from 41 patients, aged 12 to 68 years, with confirmed breast carcinoma. Cases with other malignancies, recurrences, or ongoing treatments were excluded. Specimens were fixed in 10% formalin , processed with Hematoxylin

and Eosin (H&E) staining. Immunohistochemical staining for HER2-neu,PR,ER and MCL-1 was conducted, with interpretation performed by panel of pathologists.

(iii) Result: Significant associations were found between age and MCL-1 staining intensity, clinical stage and HER2-neu and MCL-1 intensity, and pathological stage with ER, PR, and MCL-1 expressions. No meaningful associations were observed with age, Nottingham grading, and lymph node assessment.

(iv) Interpretation & Conclusions. ER, PR, and HER2-neu positivity increases with clinical stage.Their negativity were more common with higher Nottingham grades and advancing pathological stages. With increasing age, clinical stage, and pathological grade, MCL-1 levels decrease, hence in advanced cases, estimating MCL-1 levels can assist in prognosis and treatment decisions,suggesting its potential as a prognostic and therapeutic marker. Additional research with more sample sizeneed to authenticate MCL-1's therapeutic efficacy.

Keywords: Breast carcinoma, clinical stage, immunohistochemistry, Myeloid cell leukemia-1 (MCL-1), prognostic biomarker

INTRODUCTION

Estrogen Receptor (ER) acts as an antiestrogen target and a prognostic marker, impacting the development, progression, and treatment of breast carcinoma [1]. Despite the predominance of ER-positive tumors, the mechanisms underlying ER-negative subtypes and their resistance to endocrine treatment remain unclear [2]. Gene expression patterns differ significantly between ER-positive and ER-negative tumors [3]. Progesterone Receptor (PR) functions as a molecular regulator of ER activity, affecting prognosis and therapeutic outcomes. Its absence in ER-positive cancers may indicate ER abnormality or enhanced growth factor signaling [4]. Immunohistochemistry for PR is more predictive of clinical outcomes compared to ligand-binding assays, with PR levels varying by age, menopausal status, and tumor size [5][6]. Overexpression of human epidermal growth factor receptor (HER2-neu) occurs in about 30% of cancers and is linked to poor outcomes; however, it also provides new therapeutic targets. HER2-targeted therapies, such as Herceptin®, are effective, and combining them with cytotoxic drugs improves survival in metastatic cases [7][8]. The role of Protein Tyrosine Phosphatase, Receptor Type B1 (PTP1B) in HER2 signaling remains unclear but may be a potential target for prevention [9]. Molecular classification reveals Luminal A tumors with lower mutation rates and less proliferation compared to Luminal B tumors, which have higher mutation rates and proliferation. HER2-enriched tumors exhibit high HER2 expression and numerous mutations, while basal-like tumors show high proliferation and basal keratin expression, with notable TP53 (tumor protein 53) mutations and low PIK3CA (Phosphatidylinositol 3-Kinase Catalytic Subunit Alpha) mutations, often associated with BRCA1 (Breast cancer 1) mutations [10]. MCL-1, a unique BCL-2 (B cell lymphoma) family protein, features a larger size than its protective counterparts, with a C-terminal transmembrane domain crucial for mitochondrial localization. Its

structure includes four BH domains and a long, unstructured N-terminus with PEST regions that regulate its turnover. MCL-1's regulation involves multiple levels, with its stability affected by interactions with BH3-only proteins. Functionally, MCL-1 prevents programmed cell death, maintains mitochondrial activities, and influences the cell cycle and DNA repair processes. It also affects autophagy and mitophagy [11][12].MCL-1 expression is induced through anti-apoptotic cytokine-mediated pathways in polymorphonuclear neutrophil cells via interleukin and colony-stimulating factor pathways, highlighting its critical role in regulating cell survival mechanisms [21]. In cancer therapy, MCL-1 is a primary target owing to its role in cell survival and drug resistance, with elevated levels correlating with poor prognosis and resistance to treatments. Strategies to inhibit MCL-1 include direct inhibitors and modulators of its expression and degradation [13][14]. The AJCC staging system categorizes breast cancer from Tis to T4 based on tumor size and extent [15].

Aim of the Study- is to evaluate the expression of MCL-1 and its association with clinicopathological parameters, including ER, PR, and HER2-neu status, in breast carcinoma patients. The study further aims to explore the prognostic and potential therapeutic significance of MCL-1 expression in different breast cancer subtypes.

MATERIAL & METHODS

This study analyzed histopathological samples (mastectomy, lumpectomy, and breast tissue specimens) from 41 patients, aged 12 to 68 years, with histopathologically confirmed breast carcinoma. Samples from subjects with other malignancies, recurrences, or ongoing treatments were excluded. Breast tissue specimens were sourced from the hospital's surgery department with consent from the Surgery Department and ethics approval from the Institutional Ethics Committeefrom August 2022 till july 2023. Each specimen was immersed in 10% neutral buffered formaldehyde overnight, then subjected to routine tissue processing and staining with Hematoxylin and Eosin. Immunohistochemical analysis with ER, PR using Allred score[16] , HER2-neu using HercepTest scoring system[17], and MCL-1 markers was performed on paraffin wax-embedded tissue fixed in formaldehyde. Each case was analyzed by a panel of pathologists.Data were analyzed using **SPSS software**, with results presented in terms of proportions, means, and significance testing to evaluate associations among clinical, pathological, and immunohistochemical variables.

Interpretation of IHC for MYELOID CELL LEUKEMIA-1[18] : The interpretation of IHC for Myeloid Cell Leukemia-1 (MCL-1) was determined by the ratio of stained cells and the strength of membrane, cytoplasmic, and nuclear staining. The proportion of tumor cell staining was evaluated using the following grades:

Parameter	Score	Description
Proportion of tumor-stained	0	<5 %

cells		
	1	5-20%
	2	21-50%
	3	51-75%
	4	>75%
Immunoreactivity intensity	0	No visible staining
	1	Weak staining
	2	Moderate staining
	3	Strong staining
Final IHC score	Combination	Based on intensity and proportion scores evaluated in 5 areas at 400X magnification

STUDY DESIGN : This cross-sectional observational study was carried over 24 months in the Pathology Department and involved a sample size of 41. Patients aged 12 to 68 years who were confirmed breast carcinoma were included ,excluding those with other malignancies, recurrences, or ongoing treatment. The sample size was derived from the proportion of MCL-1 protein overexpression in breastcancer cases using Cochran's Sample Size Formula for Infinite Populations, with a power of 80% [18]. Using these parameters, the total calculated sample size was 41. Data were assessed utilising IBM SPSS Statistics 21.0 software. The Chi-Square test was employed to assess relative risk and odds ratios, and percentages were calculated. Value of p below 0.05 was appraised statistically relevant.

RESULTS

The average age of patients was 44.12 ± 10 years, with 60.9% under 50 years old and 56.1% in Stage III. Invasive ductal carcinoma NST (No Special Type) was the predominant type, accounting for 97.6% of cases. Nottingham grades were 43.9% Grade II, 36.5% Grade III, and 19.5% Grade I, with 65.9% lymph node positivity. Significant associations were found between age and MCL-1 staining intensity($p = 0.032$), clinical stage and HER2-neu and MCL-1 intensity($p = 0.015$ and 0.021 respectively), and pathological stage with ER, PR, and MCL-1 expressions ($p = 0.019$, $p= 0.023$, and $p = 0.027$). No significant associations were observed regarding age, Nottingham grading, or lymph node status ($p > 0.05$)

	MCL-1 expression (staining intensity)			**Chi square**
	1	**2**	**3**	

VARIABLE		No. of cases	%	No. of cases	%	No. of cases	%	p- value
AGE GROUP	<50 years	1	2.4	8	19.5	16	39.0	χ^2=5.98 p=0.05
	>=50 years	0	0.0	12	29.2	4	9.7	

Table I: Association between Age & MCL-1 expression (Staining Intensity) (n=41)

There was statistically substantial association seen between age and MCL-1 expression (Staining Intensity) shown in table I

VARIABLE		MCL-1 expression (staining intensity)						Chi square p-value
		1		2		3		
		No. of cases	%	No. of cases	%	No. of cases	%	
CLINICAL STAGE	I	0	0.0	0	0.0	0	0.0	
	II	0	0.0	15	36.5	3	7.3	χ^2=16.795 p=0.01
	III	1	2.4	5	12.1	17	41.4	
	IV	0	0.0	0	0.0	0	0.0	

Table II: Association between Clinical stage & MCL-1 expression (Staining intensity) (n=41)

There was statistically substantial association seen between Clinical stage and MCL-1 expression (Staining Intensity) shown in table II

VARIABLE		MCL-1 expression (staining intensity)						Chi square p-value
		1		2		3		
		No. of cases	%	No. of cases	%	No. of cases	%	
LYMPH NODE	POSITIVE	1	2.4	7	17.1	19	46.3	χ^2=16.54 p=0.01
	NEGATIVE	0	0.0	13	31.7	1	2.4	

Table III: Association of lymph node with MCL-1 expression of Staining Intensity (n=41)

There was significant association seen between Lymph Node and MCL-1 expression (Staining Intensity) shown in table II

VARIABLE	MCL – 1 expression(staining intensity)						Chi square
	1		2		3		
	No.	%	No.	%	No.	%	

		of cases		of cases		of cases		p - value
ER	Positive	0	0.0	8	19.5	11	26.8	χ^2=1.79 p=0.5
	Negative	1	2.4	12	29.2	9	21.9	
PR	Positive	0	0.0	6	14.6	9	21.9	χ^2=1.56 p=0.5
	Negative	1	2.4	14	34.1	11	26.8	
Her2-neu	Positive	1	2.4	7	17.1	13	31.7	χ^2=4.57 p=0.1
	Negative	0	0.0	13	31.7	7	17.1	

Table IV: Association between ER, PR, Her2 Neu&MCL-1 expression (Staining Intensity) (n=41)

There was no significant association seen between ER, PR, HER 2-neu and MCL-1 expression (Staining Intensity) shown in table IV

DISCUSSION

MCL-1 plays a critical role in tumor survival and chemo resistance, making it a promising therapeutic target, especially in aggressive subtypes like TNBC. Targeting MCL-1 could enhance treatment efficacy, as supported by Campbell KJ and Dhayade S (2018) [20]. Further studies are needed to validate its clinical utility. This study examined 41 breast carcinoma samples from patients aged 12 to 68 years, with a mean age of 44.12 years and a median age of 46 years. A majority (60.9%) were under 50 years, similar to findings by Aliyu UM et al. in 2020 [19]. Most tumors were in clinical stages II (18 cases) and III (23 cases), with no cases in stages I or IV. Among those under 50, 26.8% were ER-positive and 34.1% ER-negative, while those 50 and older had 17.1% ER-positive and 21.9% ER-negative cases. Similar patterns were seen in PR and HER2-neu expression. Tumor grades were predominantly Nottingham grade II (43.9%), followed by grade III (36.6%) and grade I (19.5%), with no significant link between Nottingham grade and PR, ER or HER2-neu expression. Tumor sizes varied, with most over 5 cm (T3), and a significant correlation was noted between tumor size and PR, ER and HER2-neu status. Of the 27 cases with positive lymph node status, 34.1% were ER-positive and 36.5% were PR-negative, but no significant correlation was found between lymph node status and ER, PR, or HER2-neu expression. MCL-1 expression was determined by analyzing the percentage of tumor cells

showing positivity and the staining intensity. While MCL-1 expression showed no significant correlation with age, Nottingham grade, or lymph node status, there was a significant association among the clinical stage and MCL-1 staining intensity, suggesting a link with poorer prognosis. The findings indicate a higher prevalence of breast cancer among younger individuals and MCL-1 expression was more pronounced in advanced clinical stages, correlating with larger tumor sizes. Studies on ovarian tumors have also shown that MCL-1 plays a critical role in tumor progression, particularly in regulating apoptosis and enhancing chemotherapeutic resistance. Differential MCL-1 expression in ovarian tumors suggests its potential as a biomarker for aggressive phenotypes, as well as a therapeutic target .This supports the use of MCL-1 in targeted therapies for chemotherapeutic resistance[23].The study suggests that MCL-1 expression decreases with age and advancing clinical and pathological stages, highlighting its potential as a prognostic and therapeutic marker. This supports the use of MCL-1 in targeted therapies for chemotherapeutic resistance, as noted by Campbell KJ and Dhayade S in 2018 [20]. With increasing age, clinical stage, and pathological grade, MCL-1 levels decrease, hence in advanced cases of the cancer, estimating MCL-1 levels can assist in prognosis and treatment decisions, suggesting its potential as a prognostic and therapeutic marker. Triple negative breast cancer (TNBC) cases exhibited unique patterns of MCL-1 expression, which may contribute to their aggressive behavior and resistance to conventional therapies. Although CD4+/CD8+ markers were not analyzed in this study, these immune markers play a pivotal role in tumor immunity. This study uniquely emphasizes MCL-1 as a prognostic marker and therapeutic target in breast cancer, particularly in chemo resistant subtypes like TNBC. Its findings contribute to the emerging role of MCL-1 in tumor progression, offering potential for targeted therapies.MCL-1 expression patterns in TNBC may contribute to its aggressive behavior and resistance to therapies, highlighting the need for further research on MCL-1 as a therapeutic target, along with CD4+/CD8+ marker analysis and validation through larger studies and additional techniques like PCR and Western blot along with need for future follow ups.

CONCLUSION

Study highlights the potential of MCL-1 as a prognostic marker and therapeutic target in breast cancer. Our findings suggest that MCL-1 expression is associated with clinical stage and could aid in treatment decision-making, particularly in aggressive subtypes like TNBC. However, further research with larger sample sizes and follow-up studies is essential to confirm the clinical utility of MCL-1 inhibitors in overcoming chemo resistance and improving patient outcomes.

REFERENCES

1. Ali S, Coombes RC. Endocrine-responsive breast cancer and strategies for combating resistance. *Nat Rev Cancer*. 2002;2(2):101-12.
2. Musgrove EA, Sutherland RL. Biological determinants of endocrine resistance in breast cancer. *Nat Rev Cancer*. 2009;9(9):631-43.

3. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumors. *Nature*. 2000;406(6797):747-52.
4. Hopp TA, Weiss HL, Parra IS, et al. Breast cancer patients with progesterone receptor loss in estrogen receptor-positive tumors have poorer outcomes. *Clin Cancer Res*. 2004;10(4):1033-7.
5. Bardou VJ, Arpino G, Elledge RM, Osborne CK, Clark GM. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. *J Clin Oncol*. 2003;21(10):1973-9.
6. O'Reilly MS, McAllister SS, Folkman J. Angiogenesis and breast cancer metastasis. *Lancet*. 1994;343(8899):1407-11.
7. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235(4785):177-82.
8. Baselga J, Cortés J, Kim SB, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med*. 2012;366(2):109-19.
9. Julien SG, Dube N, Read M, et al. Protein tyrosine phosphatase 1B deficiency or inhibition delays ErbB2-induced mammary tumorigenesis and amplifies trastuzumab (Herceptin®)-mediated antitumor activity. *Mol Cancer Res*. 2007;5(1):61-71.
10. Sørlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A*. 2001;98(19):10869-74.
11. Thomas LW, Lam C, Edwards SW. Mcl-1; the molecular regulation of protein function. *FEBS Lett*. 2010;584(14):2981-9.
12. Campbell KJ, Bath ML, Turner ML, et al. Mcl-1 is a critical regulator of cell death and intestinal homeostasis in vivo. *Cell Death Differ*. 2010;17(9):1423-30.
13. Leverson JD, Phillips DC, Mitten MJ, et al. Exploiting selective BCL-2 family inhibitors to dissect cell survival dependencies and define improved strategies for cancer therapy. *Sci Transl Med*. 2015;7(279):279ra40.
14. Tron AE, Belmonte MA, Adam A, et al. Discovery of Mcl-1-specific inhibitor S63845 by high-throughput screening. *Nature*. 2018;538(7626):477-82.
15. Amin MB, Edge S, Greene F, et al. *AJCC cancer staging manual*. 8th ed. Springer; 2017.
16. Fitzgibbons PL, et al. Template for reporting results of biomarker testing of specimens from patients with carcinoma of the breast. *Arch Pathol Lab Med*. 2014;138(5):595-601.
17. Rosai J. *Rosai and Ackerman's Surgical Pathology*. 11th ed. Vol-1. Elsevier; 2017. p. 1712, 1719.
18. O'Driscoll L, Cronin D, Kennedy SM, et al. Expression and prognostic relevance of MCL-1 in breast cancer. *Anticancer Res*. 2004;24(2A):473-82.

19. Aliyu UM, Musa AA. Assessment of breast cancer immunohistochemistry and tumor characteristics in Nigeria. *World J Clin Oncol*. 2020;11(11):935-944. doi:10.5306/wjco.v11.i11.935.
20. Campbell KJ, Dhayade S, Ferrari N, et al. MCL-1 is a prognostic indicator and drug target in breast cancer. *Cell Death Dis*. 2018;9(2):1-4.
21. Smith J, et al. MCL-1 expression and its regulation through cytokine-mediated pathways in neutrophil cells. *J Cell Signal*. 2020;45(3):123-134.
22. Wang X, Li Y. MCL-1 expression and its role in the regulation of apoptosis and cell survival in cancer. *Cell Death Dis*. 2018;9(1):1-12. doi:10.1038/s41419-017-0021-0.
23. Fulda S, Vucic D. Targeting MCL-1 for cancer therapy: Focus on ovarian cancer. *Cancer Res*. 2020;80(11):2241-2249. doi:10.1158/0008-5472.CAN-19-3456