ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF PATHOLOGY

Association of Ki-67 Expression with Clinicopathological Parameters and Prognostic Significance in Breast Cancer at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

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Declaration

I, Dr. Tensae Tesfaye, hereby declare that the research proposal entitled:

"Association of Ki-67 Expression with Clinicopathological Parameters and Prognostic Significance in Breast Cancer at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia Cross Sectional Analytic Study "is my original work and has been prepared in accordance with the academic and ethical guidelines of Tikur Anbessa Specialized Hospital (TASH) and was fully undertaken by me under the guidance of my advisor *Dr. Amanuel Damie*. To the best of my knowledge, this proposal has not been submitted in part or in full to any other institution or for any other academic qualification.

I affirm that all sources of information, data, and ideas used in the development of this proposal have been acknowledged and referenced. I also confirm that ethical approval was obtained prior to the commencement of the study, and all necessary ethical standards, including patient confidentiality and informed consent (where applicable), were strictly adhered to.

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Abstract

Background: Breast cancer is the most frequently diagnosed and is one of the leading cause of cancer related deaths among women worldwide, including Ethiopia. One of the major prognostic factors is the degree of tumor cell proliferation, and Ki-67, a nuclear protein associated with cellular proliferation has attracted much attention for its potential use in delineating therapeutic strategies as well as in outcome prediction. In areas where resources are scarce and gene assays are mostly inaccessible, markers such as Ki-67 that are regulated are of utmost importance for the proper stratification of patients.

Objective: To evaluate the prognostic significance of Ki-67 expression in patients with breast cancer and its association with clinicopathological parameters and one-year outcomes at Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia.

Methods: An institutional-based retrospective chart review was performed for 130 patients with histologically confirmed breast cancer and immunohistochemistry (IHC) results at Pathology department, Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia. Data on sociodemographic characteristics, tumor grade, stage, lymph node status, and IHC markers (ER, PR, HER2, and Ki, 67) were collected. KI-67 expression was divided into low (<14%) or high (14%) levels. Data analysis was done using SPSS version 27. Bivariate and multivariate logistic regression were used to identify factors independently associated with high KI-67 expression and its association with one-year clinical outcomes.

Results: The study found that 60.8% of patients showed high Ki-67 expression which reached or exceeded the 14% threshold. The analysis found that high Ki-67 levels were significantly associated with ER/PR negativity, HER2 positivity, increased tumor grade, advanced pathologic stage, lymph node spreading and aggressive molecular subtype patterns. The multivariate analysis identified HER2 positive status with AOR = 11.35 and 95% CI = 3.02-42.73 and $p < 0.001$ and lymph node involvement with AOR = 5.85 and 95% CI = 1.70-20.17 and $p = 0.005$ and molecular subtype with AOR = 4.98 and 95% CI = 2.41-10.30 and $p < 0.001$ as independent factors that linked to high Ki-67 expression.During the one year follow-up, patients with a high proliferation index of the Ki-67 tumor cell marker had higher rates of cancer recurrence, disease progression, and mortality.

Conclusion: High Ki-67 expression is very common in breast cancer patients in Ethiopia and its independent association has been found between the aggressive tumor features and poor short-term outcomes. This study strongly advocates for the standard inclusion of Ki-67 in pathology reports for the betterment of the prognostic evaluation and direction of therapy decisions, especially in areas where the access to molecular profiling is very limited.

Keywords: Ki-67, Breast Cancer, Clinicopathological Parameters, Prognosis, Ethiopia, Tikur Anbessa Specialized Hospital.

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Abbreviation and acronym

Abbreviation	Full Term
BC	Breast Cancer
DFS	Disease-Free Survival
ECE	Extracapsular Extension
ER	Estrogen Receptor
FFPE	Formalin-Fixed, Paraffin-Embedded
H&E	Hematoxylin and Eosin
HER2	Human Epidermal Growth Factor Receptor 2
HR	Hormonal Receptor / Hormone Receptor
IHC	Immunohistochemistry
IRB	Institutional Review Board
Ki-67	A nuclear protein associated with cellular proliferation
LI	Labeling Index
LVI	Lymphovascular Invasion
MF/MC	Multifocality/Multicentricity
MIB-1	Mouse Monoclonal Antibody Against Ki-67
OS	Overall Survival
PR	Progesterone Receptor
ROC	Receiver Operating Characteristic
SPSS	Statistical Package for the Social Sciences
TNBC	Triple-Negative Breast Cancer
WHO	World Health Organization

1.Introduction

1.1 Background

Breast cancer is the most commonly diagnosed cancer in females and the is one of the leading cause of death worldwide. Incidence is increasing in low- and middle-income countries in recent decades. In Ethiopia, the incidence of breast cancer is steadily increasing, and it has become the most commonly diagnosed cancer in the country. (1)

Breast cancer risk is influenced by a range of genetic, hormonal, reproductive, and lifestyle factors. Key risk factors include family history, nulliparity, late age at first childbirth, early menarche and or late menopause, and history of benign breast disease. (2)

In regards the histologic subtype of breast cancer in Ethiopia, the most common one is ductal carcinoma, followed by lobular carcinoma. (3)

The patient's age, tumor size, lymph node status, histological type, histological grade, lymphovascular invasion, hormonal receptor status, human epidermal growth factor receptor 2 (Her2neu) expression, and Ki-67 labeling index for proliferation rate are the different clinicopathologic parameters that can help determine the appropriate management strategy in these patients.(4)

One of the most important prognostic indicators in breast cancer is tumor cell proliferation. Ki-67, a nuclear protein expressed during all active phases of the cell cycle except G0, serving as a a reliable marker of proliferation. Its role as a proliferation index has attracted significant interest, and it has been studied over the past decade for its potential to guide prognosis and therapeutic decisions.(5,6)

Estrogen receptor (ER) and progesterone receptor (PR) are nuclear receptors that bind to estrogen and progesterone.(7,8) Staining can also be seen in normal breast acini which serve as important internal controls during testing. However, staining in normal tissue can be heterogeneous and may vary with the menstrual cycle. (8)

ER regulates the synthesis of PR, making ER-positive/PR-negative tumors relatively common, whereas ER-negative/PR-positive tumors are unusual and may indicate a false-positive result. Although ER and PR are considered weakly favorable prognostic markers, with expression generally correlating with better differentiation and their greatest value lies in their strong predictive significance, as tumors expressing these receptors are likely to respond to hormonal therapies, especially tamoxifen. (7,8)

HER2/Human epidermal growth factor receptor 2 is Routinely assessed in all primary invasive breast cancer and metastatic / recurrent breast cancer to inform eligibility for anti-HER2 directed targeted therapy by detecting the presence of protein overexpression by evaluation of the membranous staining in the tumor cells.(9)

At the 12th St. Gallen International Breast Cancer Conference (2011), the Expert Panel introduced a revised classification system for therapeutic decision-making in breast cancer. This system recommended the use of clinicopathological markers—such as hormone receptor (ER/PR), HER2, and Ki-67—as practical surrogates for molecular subtyping, replacing the need for gene expression profiling. Based on these markers, breast cancer is categorized into four molecular subtypes: Luminal A-like(HR+ HER2−, low Ki-67), Luminal B-like(HR+ HER2+ or HR+ HER2− with high Ki-67), HER2-enriched (HR− HER2+), and Triple-Negative (HR− HER2−). This classification helps guide prognosis and treatment decisions. (10)

1.2 Statement of the problem

The rising burden of breast cancer represents a major global public health concern. In 2022, around 2. 3 million women were diagnosed with breast cancer and about 670,000 women died from the disease all over the world. Female breast cancer ranks 2nd as a cause of cancer-related deaths globally with only lung cancer ahead of it.(11,12)

In Ethiopia, the burden of breast cancer is increasing rapidly. Breast cancer is now the leading cause of cancer illness among adult women in Ethiopia it makes up for nearly one-third of all female cancer cases in the country. In 2022, an estimated 16,904 new cases were reported, accounting for (32.1%) all female cancers. (13)

Ki-67 index is a very useful to determine the prognosis. And multiple studies have been done studying the relationship of the Ki-67 score with clinicopathological parameters in invasive breast carcinomas. Thus, having a standardized of interpretation of Ki-67 proliferative index and cut-off value for scoring will improve the demonstration of the prognostic significance of Ki-67 in invasive breast carcinomas.(14)

In addition to conventional clinicopathological parameters like age at presentation, tumor size, lymph node status, lymphovascular invasion, tumor grade and tumor staging that have been traditionally used to determine prognosis in patients with breast cancer, uncontrolled proliferation of tumor cells is one of the crucial hallmark of cancer and is one of the essential element for the therapeutic choice in breast cancer patients. (15)

To the best of our knowledge, no previous study has been conducted on the prognostic significance of Ki-67 and its association with clinicopathological parameters of breast cancer in Ethiopia.

1.3 Significance of the study

This study aims to clarify and enhance the understanding of the prognostic relevance of Ki-67 in breast cancer and its relationship with commonly evaluated clinicopathological parameters . Understanding these associations can help refine prognostication and guide therapeutic decisions particularly in hormone receptor-positive breast cancer where Ki-67 guides the choice between hormonal therapy and chemotherapy, especially in resource-limited countries like Ethiopia where genomic assays may not be feasible there by providing evidence for integrating Ki-67 into routine clinical practice for treatment planning.

It also provides insight into biological behavior and aggressiveness of tumors by risk stratification and prognostic assessment in breast cancer patients.

It will contribute to standardization of Ki-67 cutoffs, aiding in global harmonization of breast cancer biomarker reporting.

2. Literature review

A retrospective study conducted in Amasya University, Turkey, demonstrated that the correlations between the Ki-67 score and clinicopathological parameters remained consistent regardless of whether the cut-off value was set at 14% or 20%. A high Ki-67 score was significantly associated with higher tumor grade, the presence of lymphovascular invasion (LVI), and estrogen receptor (ER) negativity. Furthermore, Ki-67 scores were found to be higher in cases classified as the Luminal B subtype and lower in Luminal A cases, compared to other molecular subtypes.(14)

A retrospective study of 2,250 primary breast cancer patients (2004–2020) at Università di Roma La Sapienza, Ospedale Sant'Andrea (Rome, Italy) found that, in luminal tumors, a Ki-67 cut-off of 20% more reliably distinguishes low- and high-risk patients, helping identify those eligible for adjuvant chemotherapy.(16)

A cross-sectional study conducted at Maharaj Nakorn Chiang Mai Hospital in Northern Thailand included 507 breast cancer patients diagnosed between January 2013 and December 2015. The study identified 30% as the optimal Ki-67 cut-off point in this population. Among (HR+) patients, those with Ki-67 levels above 30% had a significantly lower 8-year survival rate ($p < 0.001$). However, Ki-67 levels did not show a statistically significant difference in overall survival (OS) among patients with HER2-positive, triple-positive, or triple-negative subtypes.(17)

A study conducted at Yenepoya University, Mangalore (Karnataka, India) involving 70 breast cancer patients found that Grade II tumors were the most prevalent, with the majority showing <15% Ki-67 expression, though without statistical significance. Ki-67 expression was significantly higher in tumors >5 cm (47.6%) compared to those <5 cm (14.3%), with a p-value of 0.003. Most patients presented with pT2 tumors, showing <15% Ki-67 expression; however, no significant association was found between Ki-67 and pT category ($p > 0.05$). Ki-67 expression was not significantly associated with lymph node status. Among molecular subtypes,

Basal-like was most common, followed by Luminal A and Luminal B. High Ki-67 expression was most frequent in Luminal A (32.1%), followed by Basal-like (28.3%), HER2-enriched (25.5%), and Luminal B (15.1%). A significant association was observed between Ki-67 expression and molecular subtype (p = 0.007).(5)

A study conducted at Kumamoto City Hospital in Japan analyzed 4,701 patients with primary breast cancer diagnosed between 1987 and March 2013. The Ki-67 proliferation index was evaluated by counting at least 500 tumor cells, including the hotspot area, and categorized into three groups: <20%, ≥20%, and ≥50%. Higher Ki-67 index values were significantly associated with higher tumor grade, negative ER/PR status, positive HER2 status, shorter disease-free interval, and increased risk of recurrence.(18)

A study from Pakistan reported significant associations between the Ki-67 index and tumor grade, PR status, HER2neu positivity, and lymph node involvement, but not with ER status or tumor size. Ki-67 showed an inverse relationship with PR positivity and a direct correlation with HER2neu expression. Notably, tumors with high Ki-67 (>30%) had lower HER2neu positivity and lymph node metastasis compared to those with intermediate Ki-67 (16–30%), suggesting that some high-proliferation tumors may have a better prognosis. The study supports using Ki-67 alongside other markers and recommends classifying it into low, intermediate, and high-risk groups to guide adjuvant chemotherapy and prognostic decisions.(19)

Data from 308,680 women in England (2009–2016) showed that Ki-67 is a strong prognostic marker for breast cancer mortality in ER-positive, HER2-negative early invasive breast cancer. Ki-67 remained prognostic above ~30%, and strongly so above 40%, even without adjusting for tumor size or nodal status—highlighting its potential role in the neoadjuvant setting. These findings support the IKWG's 30% cut-off over the 10% threshold used in the PREDICT tool.(20)

A cross-sectional analytical study of 220 breast cancer patients at Tabriz University of Medical Sciences (Iran) found no significant association between Ki-67 expression and patient age, tumor size, or grade. A marginally significant relationship was observed with lymph node status. Significant associations were found between Ki-67 and both ER and PR status. However, the

study emphasized the need for more reliable prognostic markers to guide optimal treatment . (21)

A prospective study from India, published in October 2023 and involving 127 female breast cancer patients, found that the Ki-67 index was positively correlated with pathological stage (p=0.025), tumor grade (p=0.0001), and molecular subtype (p=0.0001). It showed an inverse association with ER and PR status (both p=0.0001), but no significant correlation with tumor size (p=0.08), nodal status (p=0.06), age (p=0.892), or HER2 status (p=0.287). These findings support Ki-67 as a relevant prognostic marker, particularly in relation to grade, stage, and hormone receptor . (22)

A case-control study conducted in Iraq found that median Ki-67 expression scores were significantly higher in newly diagnosed breast cancer patients compared to controls ($p < 0.001$). Ki-67 levels increased with tumor size and grade, showed a significant negative correlation with estrogen receptor expression, and a positive correlation with HER2 expression. (23)

A study at Kumamoto City Hospital, Japan, found that a higher Ki-67 index was significantly associated with larger tumor size, younger age, lymph node positivity, higher nuclear grade, negative ER/PR status, p53 overexpression, and HER2 positivity. Tumors in older patients (≥65 years) had lower Ki-67 levels, while no significant difference was observed between patients aged 36–50 and 50–65 years. (24)

A study from Ireland highlighted that while Ki-67 indices offer a precise measure of breast cancer cell proliferation and are widely used in histopathology, their clinical reliability is limited by inconsistent assessment methods, absence of standardized guidelines, and variable use of multigene panels incorporating Ki-67. (25)

A study from China found that higher ER and PR levels were associated with lower Ki-67 scores, while higher HER2 levels correlated with higher Ki-67 scores. Ki-67 increased with tumor size during early breast cancer stages but plateaued at more advanced stages, possibly due to limited blood supply and nutrients restricting further tumor proliferation.(26)

A study from India found a significant association between Ki-67 expression and both ER positivity and HER2neu overexpression in breast cancer patients, with a high proliferative rate observed in these groups. No significant correlation was noted with other clinicopathological parameters. The study emphasized the need for a standardized Ki-67 cut-off to improve the generalizability of findings.(4)

3. Objectives

3.1 General objectives

To evaluate the prognostic significance of Ki-67 expression in patients with breast cancer and its association with the clinicopathologic parameters and outcomes at Tikur Anbessa Specialized Hospital (TASH) Addis Ababa, Ethiopia from January 1, 2023, to Dec 30, 2024.

3.2 Specific objectives

1. To identify the prognostic significance of Ki-67 in patients with breast cancer.
2. To determine the association of Ki-67 with clinico-pathological parameters such as tumor grade, lymph node status, LVI and disease recurrence and with hormone receptor status (ER, PR) and HER2 expression.
3. To assess Ki-67 index across molecular and histological subtypes of breast cancer
4. To determine the association of Ki-67 with one year outcome of patients with breast cancer .

4. Method and materials

4.1 Study area

The study was conducted at TASH, located in Addis Ababa, the Capital city of Ethiopia. TASH is the largest referral hospital in Ethiopia that was originally founded as Prince Mekonnen Memorial Hospital in 1961 and was renamed Tikur Anbessa Hospital in **1976**, which is located at Lideta sub city. It has served as a main teaching hospital, administered by Addis Ababa University which has currently gained its autonomy, with an annual 25,000 patients visit per year. The Pathology Department at Tikur Anbessa Hospital was opened in **1965**, marking the establishment of the first anatomic pathology department in Ethiopia. This facility also launched Ethiopia's first pathology residency program in 1981, which has since trained a significant number of pathologists for the country and currently has 16 specialists with 13 residents currently enrolled in the program.

4.2 Study design and period

The research design used for this study was Retrospective chart review of histologically confirmed breast cancer patients with immunohistochemistry results conducted from January 1, 2023, to Dec 30, 2024 among Breast cancer patients. Data was collected from October 1, 2025 to December 30 2025 through a standard checklist by a data collector. Additionally, basic demographic characteristics of patients, previous medical, surgical history and treatment (chemotherapy) history was checked to identify their impact on the study.

4.3 Population

4.3.1 Target population

- All Breast cancer patients at Tikur Anbessa Specialized Hospital (TASH) Addis Ababa, Ethiopia.

4.3.2 Source population

All patients with confirmed breast carcinoma at the department of pathology at Tikur Anbessa Specialized Hospital (TASH) are the source population.

4.3.3 Study population

All Breast cancer patients with histologically confirmed breast carcinoma at Tikur Anbessa Specialized Hospital (TASH) which also fulfilled the inclusion criteria are the study population.

4.4 Eligibility criteria

4.4.1 Inclusion criteria

- Histologically confirmed cases of invasive breast carcinoma.
- Patients with available immunohistochemistry results for ER, PR, HER2, and Ki-67.
- Age 18 years and above.

4.4.2 Exclusion criteria

- Incomplete records
- Recurrent disease
- Patients who received neoadjuvant therapy prior to biopsy.

4.5 Sample size determination and Sampling technique

4.5.1 Sample size determination

The sample size was calculated by using a single proportion formula with assumptions of 95% confidence interval, 0.05 margin of error, since there is no previous study on the area the population proportion of 50% will be taken then 10% of nonresponse rate was added and finite population adjustment was done as our study population size is less than 10,000.

$$N = \frac{z^2 \times p(1-p)}{d^2}$$

$$N = \frac{1.96^2 \times 0.5(1-0.5)}{0.05^2}$$

N=384

- n = sample size.
- $Z_{\alpha/2}$ = level of confidence (95%)
- d= desired precision (Margin of Error) – 0.05
- P= the proportion

$$n_{adj} = \frac{n}{1 + \frac{n}{N}}$$

- where n adj = Adjusted sample size
- n = calculated sample size (384)
- N = study population (180)

n_{adj}= = 122 was the sample size of the study. With an additional 10% of the non-respondent rate the final sample size was 134.

4.5.2 Sampling method

All patients who meet the inclusion criteria are included in the study.

4.6Study variable

4.6.1 Independent variable

- ✓ Age
- ✓ Tumor size
- ✓ Lymph node status
- ✓ Histological type
- ✓ Histological grade
- ✓ Lymphovascular invasion
- ✓ Hormonal receptor status
- ✓ Human epidermal growth factor receptor 2 (Her2neu) expression
- ✓ Clinical outcome

4.6.2 Dependent variable

- ✓ Ki-67

4.7 Operational definition

- **Estrogen receptor (ER) and progesterone receptor (PR)** are nuclear receptors that bind estrogen and progesterone, exerting their effects directly in the nucleus. (7,8)
    - *A critically important statement from the NIH Consensus Conference of December 2000 stated that "any nuclear expression of HRs should be regarded as a positive result and render a patient eligible for hormonal therapy.". The current CAP/ASCO guidelines on HR testing also recommend ≥1% of positive cells as a positive result for ER and PR.*(8)

- ***HER2/Human epidermal growth factor receptor 2-*** *HER2 signaling activates multiple major signaling pathways (Ras / Raf / MEK / MAPK and PI3K / AKT) that promote cellular proliferation and antiapoptosis and HER2 gene amplification results in HER2 protein overexpression.*(9)
    - Evaluation of the membranous staining in the tumor cells is done.
- ***Ki-67-****signals cell growth. It is a highly labile, nonhistone nuclear protein that is expressed during G1 ,S, G2, and M phases of cell cycle, after which it is quickly broken down at the end of M phase and is not found in G0 and early G1 cells. This protein is a useful to measure the developmental levels of a cell population as it exists in all types of replicating cells.*
- *Moreover, luminal-like breast tumors can be divided into luminal A and luminal B groups using the* Ki-67. ***Luminal A like*** *tumors are estrogen receptor and progesterone receptor (ER/PR) positive and human epidermal growth factor receptor 2 (HER2/neu) negative with low* Ki-67 *protein(*Ki-67 *LI <14%).* ***Luminal B like*** *tumors are estrogen receptor and progesterone receptor (ER/PR) positive and human epidermal growth factor receptor 2 (HER2/neu) variable with intermediate to high* Ki-67 *index(*Ki-67 *LI ≥14%).*(6,8)
- Normal breast tissue demonstrates very low levels of Ki-67 index (< 3%)(28)

4.8 Data collection tool and procedure

Development of the items of the data collection checklist was accomplished by adapting valid tools from the literature review. It had closed-ended and some open-ended questions including of Socio-demographic characteristics and, basic information. The data collection checklist is prepared in English.

Data was collected from patient's medical record, laboratory, biopsy results and immunohistochemistry result. The Data collection period was planned between October 1 2025 to December 30 2025 but was extended to the first 2 weeks of January 2026..

Data was collected by a the principal invertigator after receiving training on the study and data collection tools. The completeness of the data was checked and evaluated by the data evaluator before completion of data.

4.9 Data quality control

The data collection checklist was articulated clearly to be understood by the data collector. The trained data collector was largely responsible for collecting data, and before entering the information into the SPSS version 27th, the responses was reviewed by the data evaluator for completeness of the data. 5% of the study population underwent a pretest.

4.10 Data processing and analysis

The completed data was coded and entered into version 27th SPSS for analysis by the principal investigator. The descriptive data was described using mean with standard deviation, median with inter-quartile range, to characterize the study population using socio-demographic and background characteristics.

Two categorical variables were analyzed using the chi-square test and Fisher exact test to check for correlation and uni-variate analysis was done to check and measure level of association with each independent variables to the dependent variables and variables with p-values <0.25 were taken to the multi-variable logistic regression model to sort out cofounding effect with one another and those variables with p-value of <0.005 were taken as significant.

4.11 Ethical consideration

A formal letter of approval was obtained from the Department of Pathology, College of Health Sciences, Addis Ababa University to conduct this study. The research was initiated only after receiving ethical clearance from the Ethics committee of the Department. Data collection commenced following the issuance of an official letter of permission from the relevant authorities.

To ensure confidentiality, the data collection checklist was filled out anonymously, and no personal identifiers were included in the data collection tool. All collected data were securely stored and analyzed solely by the principal investigator. Participants' identities were not disclosed to any third party, and the information obtained was used strictly for research purposes.

5. Results Chapter

5.1 Sociodemographic Characteristics

Out of 130 patients, the majority were female (96.9%), and only 3.1% were male. The mean age at diagnosis was 47.8 years (SD ±10.6), median 48 years (IQR 40–55). Most patients were postmenopausal (46.9%), followed by premenopausal (44.6%) while 8.5% were unknown/not applicable.

Table 1 Sociodemographic characteristics of study participants

Variable	**Category**	**Frequency (n)**	**Percentage (%)**
Sex	Female	126	96.9
	Male	4	3.1
Age at diagnosis	<40	44	33.8
	40–49	33	25.4
	50–59	32	24.6
	60+	21	16.2
Mean ± SD	–	47.8 ± 10.6	–
Median (IQR)	–	48 (40–55)	–
Menopausal status	Pre-menopausal	58	44.6
	Post-menopausal	61	46.9
	Unknown/Not applicable	11	8.5

5.2 Tumor Characteristics

Most tumors were unilateral, with right sided predominance (53.1%). Tumor size varied, with the majority between 20–50 mm (39.2%). Stage II was most common (36.9%), followed by Stage IV (19.2%). Histologically, invasive breast carcinoma NST predominated (90.8%). Grade II and III tumors were frequent, while Grade I accounted for 9.2%.

Table 2 Tumor characteristics

Variable	**Category**	**Frequency (n)**	**Percentage (%)**
Tumor laterality	Right	69	53.1
	Left	53	40.8
	Bilateral	6	4.6
	Unknown	2	1.5
Tumor size	>1–5 mm	2	1.5
	>5–10 mm	4	3.1
	>10–20 mm	15	11.5
	>20–50 mm	51	39.2
	>50 mm	21	16.2
	Unknown	37	28.5
Histological type	Invasive Breast Carcinoma NST	118	90.8
	Lobular carcinoma	4	3.1
	Mucinous carcinoma	4	3.1
	Mixed ductal/lobular	2	1.6
Tumor grade	Grade I	12	9.2
	Grade II	44	33.8
	Grade III	42	32.3
	Unknown	32	24.6

5.3. Pathologic stages

Stage II was most common (48, 36.9%), followed by Stage IV (25, 19.2%) (Figure 1).

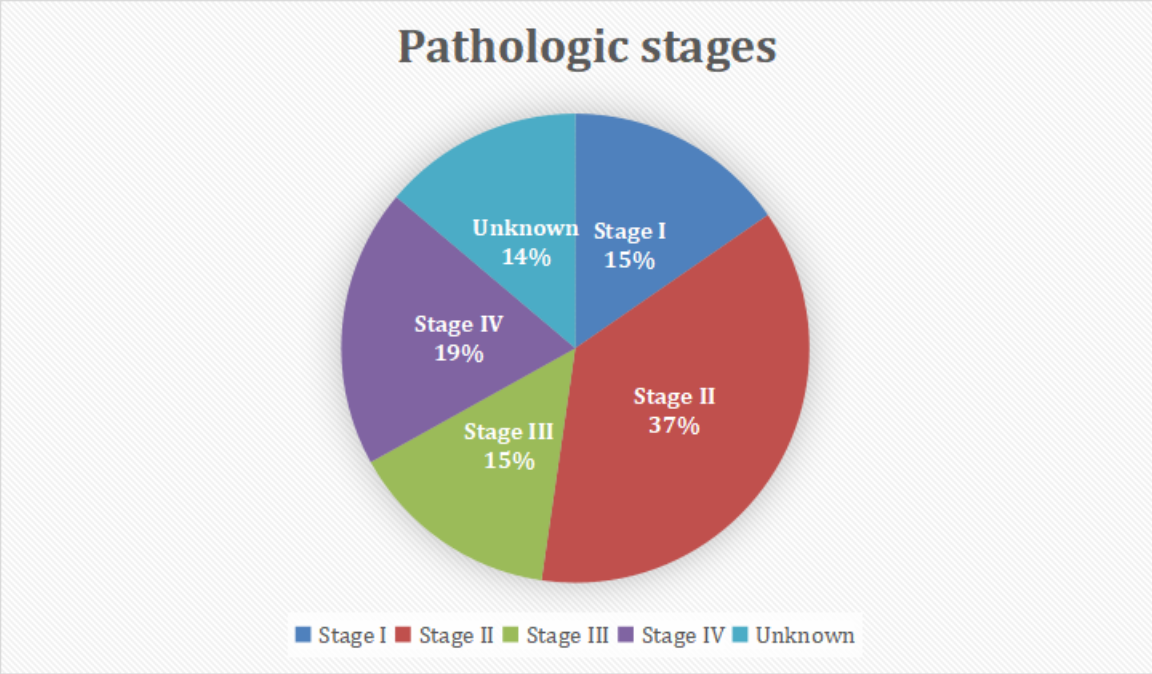


Figure 1 Pathologic stages

5.4 Pathological Features

Lymph node involvement was seen in 58.5% of patients. Distant metastasis was present in 13.8%. Lymph vascular invasion was reported in 41.5%, while perineural invasion was rarely reported (4.6%) (Table 3).

Table 3 Pathological features

Variable	Category	Frequency (n)	Percentage (%)
Lymph node involvement	Positive	76	58.5
	Negative	33	25.4
	Not assessed	21	16.1
Distant metastasis	Yes	18	13.8

	No	88	67.7
	Not known	24	18.5
Lymphovascular invasion	Present	54	41.5
	Absent	30	23.1
	Not reported	46	35.4
Perineural invasion	Present	6	4.6
	Absent	19	14.6
	Not reported	105	80.8

4. Hormone Receptor and HER2 Status

ER positivity was seen in 73.1% of cases, PR positivity in 57.7%, and HER2 positivity in 22.3% while only 3.2% were Equivocal (Table 4).

Table 4 Hormone receptor and HER2 status

Marker	**Category**	**Frequency (n)**	**Percentage (%)**
ER	Positive	95	73.1
	Negative	35	26.9
PR	Positive	75	57.7
	Negative	55	42.3
HER2	Positive	29	22.3
	Negative	97	74.6
	Equivocal	4	3.1

5.5 Ki-67 Proliferation Index

The Ki-67 index ranged from 5 to 83, with a mean of 32.4 ±14.8. Using a 14% cut-off, 60.8% of patients had high Ki-67 expression (Table 5, Figure 2)

Table 5 Ki-67 proliferation index

Statistic	Value
Minimum	5
Maximum	83
Median (IQR)	30 (20–40)
Low Ki67 (<14%)	51 (39.2%)
High Ki67 (≥14%)	79 (60.8%)

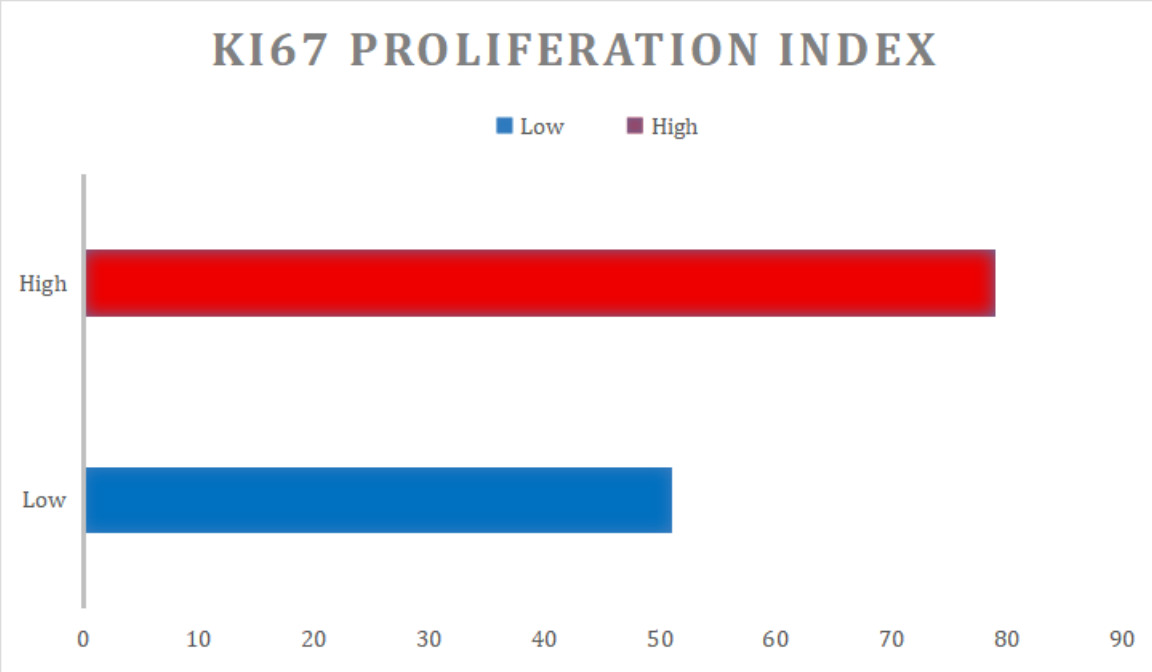


Figure 2 Ki-67 proliferation index

5.6 Surrogate for Molecular Subtypes

Luminal A like was the most common subtype (33.8%), followed by Luminal B like (33.1%) ,Triple-negative (20.0%), and HER2-enriched (13.1%) Table 6.

Table 6 Molecular Subtypes

Subtype	Frequency (n)	Percentage (%)
Luminal A like	44	33.8
Luminal B like	43	33.1
HER2-enriched	17	13.1
Triple-negative	26	20.0

5.7 Treatment Modalities

Mastectomy was the predominant surgical modality (67.7%). Chemotherapy was administered for 84.6% of patients, radiotherapy to 29.2%, and hormonal therapy to 54.6%.

Table 7 Treatment modalities

Treatment type	Category	Frequency (n)	Percentage (%)
Surgery	Mastectomy	88	67.7
	Lumpectomy	11	8.5
	Core biopsy	28	21.5
	Mastectomy + core biopsy	3	2.3
Chemotherapy	Yes	110	84.6
	No	9	6.9
	Unknown	11	8.5
Radiotherapy	Yes	38	29.2
	No	80	61.5
	Unknown	12	9.2
Hormonal therapy	Yes	71	54.6
	No	46	35.4
	Unknown	13	10.0

5.8 Patient Outcomes at One Year

At one year, 50% of patients were disease free. Recurrence was observed in 22.7% (local, regional, distant), progressive disease in 9.2%, new primary breast cancer in 0.8%, and death in 10.8%.

Table 8 Patient Outcomes at One Year

Outcome category	Frequency (n)	Percentage (%)
Disease-free	65	50.0
Local recurrence	12	9.2
Regional recurrence (lymph nodes)	5	3.8
Distant recurrence / Metastatic	11	8.5
Progressive disease	12	9.2
New primary breast cancer	1	0.8
Died	14	10.8
Unknown / Not assessed	10	7.7

5.9 Association of Ki-67 Expression with Clinicopathological Parameters and Patient Outcomes

To identify the association between Ki-67 expression and predictive variables, a bivariate logistic regression analysis was first performed for all sociodemographic and clinicopathological parameters. Variables with a p- value less than 0.2 were considered candidates for multivariable analysis (Table 9). The candidate variables were entered into SPSS version 27 using the enter method.

The application of the 14% cut-off demonstrated that 60.8% of patients had for high Ki-67 levels. The bivariate analysis discovered a significant link between high Ki-67 levels and ER negativity, PR negativity, HER2 positivity, advanced tumor grade, advanced cancer stage, lymph node involvement, and specific aggressive molecular subtypes (Luminal B like, HER2-enriched, triple-negative). The one-year patient outcomes showed distinct patterns because high Ki-67 levels increased the likelihood of recurrence, progression, and death.

The multivariable logistic regression model identified HER2 positivity (AOR = 11.35, 95% CI = 3.0242.73, $p < 0.001$), lymph node involvement (AOR = 5.85, 95% CI = 1.7020.17, $p = 0.005$), and molecular subtype (AOR = 4.98, 95% CI = 2.4110.30, $p < 0.001$) as the only factors that independently predicted high Ki67 expression. The analysis showed that age prediction ability

decreased to non-significance after adjustments for other variables (AOR = 0.84, 95% CI = 0.521.34, p = 0.460). The statistical significance of ER and PR status together with other variables disappeared after adjustment because molecular subtype and HER2 status both shared prognostic value(Table 9).

Table 9 Factors associated with Ki-67 expression with sociodemographic, clinicopathological parameters, and one- year outcomes

Variable / Category	Ki-67 **Low** (n, %)	Ki-67**High** (n, %)	COR (95% CI)	p value (COR)	AOR (95% CI)	p value (AOR)
Sex						
Female	50 (43.1%)	66 (56.9%)	Ref	–	Ref	–
Male	1 (25.0%)	3 (75.0%)	2.73 (0.28–26.93)	0.37	0.00 (unstable)	1.000
Age at diagnosis						
<40	17 (38.6%)	27 (61.4%)	Ref	–	Ref	–
40–49	14 (42.4%)	19 (57.6%)	0.86 (0.36–2.05)	0.73	0.86 (0.36–2.05)	0.73
50–59	15 (46.9%)	17 (53.1%)	0.71 (0.30–1.68)	0.44	0.71 (0.30–1.68)	0.44
60+	15 (71.4%)	6 (28.6%)	0.25 (0.09–0.72)	0.01	0.84 (0.52–1.34)	0.460
Menopausal status						
Premenopausal	25 (43.1%)	33 (56.9%)	Ref	–	Ref	–
Postmenopausal	32 (52.5%)	29 (47.5%)	0.69 (0.34–1.40)	0.31	2.03 (0.16–25.63)	0.585
Not applicable	2 (40.0%)	3 (60.0%)	1.14 (0.18–7.10)	0.89	0.34 (0.03–3.97)	0.386
Unknown	2 (33.3%)	4 (66.7%)	1.52 (0.26–8.80)	0.64	1.52 (0.26–8.80)	0.64
ER status						
Positive	55 (57.9%)	40 (42.1%)	Ref	–	Ref	–
Negative	6 (17.1%)	29 (82.9%)	6.65 (2.52–17.51)	<0.001	1.11 (0.18–6.78)	0.907
PR status						
Positive	46 (61.3%)	29 (38.7%)	Ref	–	Ref	–
Negative	15 (27.3%)	40 (72.7%)	4.23 (1.99–8.99)	<0.001	3.22 (0.76–13.68)	0.113
HER2 status						
Negative	52 (53.6%)	45 (46.4%)	Ref	–	Ref	–
Positive	7 (24.1%)	22 (75.9%)	3.60 (1.40–9.20)	0.007	11.35 (3.02–42.73)	<0.001
Equivocal	2 (50.0%)	2 (50.0%)	1.20 (0.20–7.00)	0.85	1.20 (0.20–7.00)	0.85
Tumor grade						
Grade I	10 (83.3%)	2 (16.7%)	Ref	–	Ref	–
Grade II	25 (56.8%)	19 (43.2%)	3.80 (0.80–17.70)	0.09	1.27 (0.64–2.51)	0.489
Grade III	10 (23.8%)	32 (76.2%)	16.00 (3.50–72.50)	<0.001	1.27 (0.64–2.51)	0.489

Unknown	16 (50.0%)	16 (50.0%)	5.00 (1.00–24.00)	0.05	5.00 (1.00–24.00)	0.05
Pathologic stage						
Stage I	13 (65.0%)	7 (35.0%)	Ref	–	Ref	–
Stage II	25 (52.1%)	23 (47.9%)	1.70 (0.60–4.90)	0.29	1.70 (0.60–4.90)	0.29
Stage III	8 (42.1%)	11 (57.9%)	2.60 (0.80–8.10)	0.11	0.85 (0.51–1.42)	0.534
Stage IV	7 (28.0%)	18 (72.0%)	4.80 (1.40–16.00)	0.01	4.80 (1.40–16.00)	0.01
Unknown	8 (44.4%)	10 (55.6%)	2.30 (0.70–7.50)	0.16	2.30 (0.70–7.50)	0.16
Lymph node involvement						
Negative	32 (59.3%)	22 (40.7%)	Ref	–	Ref	–
Positive	19 (28.4%)	48 (71.6%)	2.36 (1.16–4.81)	0.018	5.85 (1.70–20.17)	0.005
Not assessed	–	–	–	–	0.76 (0.17–3.32)	0.716
Molecular subtype						
Luminal A like	44 (100.0%)	0 (0.0%)	Ref	–	Ref	–
Luminal B like	1 (2.3%)	42 (97.7%)	∞	<0.001	4.98 (2.41–10.30)	<0.001
HER2 enriched	3 (17.6%)	14 (82.4%)	∞	<0.001	5.40 (2.53–11.53)	<0.001
Triple-negative	3 (11.5%)	23 (88.5%)	∞	<0.001	–	–
Patient outcome (1 year)						
Disease free	38 (58.5%)	27 (41.5%)	Ref	–	Ref	–
Local recurrence	4 (33.3%)	8 (66.7%)	2.80 (0.80–9.50)	0.11	2.80 (0.80–9.50)	0.11
Regional recurrence	0 (0.0%)	5 (100.0%)	∞ (complete separation)	<0.001	∞	<0.001
Distant recurrence	4 (36.4%)	7 (63.6%)	2.50 (0.70–8.60)	0.14	2.50 (0.70–8.60)	0.14
Progressive disease	4 (33.3%)	8 (66.7%)	2.80 (0.80–9.50)	0.11	2.80 (0.80–9.50)	0.11
New primary breast cancer	0 (0.0%)	1 (100.0%)	∞ (complete separation)	<0.001	∞	<0.001
Died	6 (42.9%)	8 (57.1%)	1.90 (0.60–6.00)	0.27	1.90 (0.60–6.00)	0.27

6. Discussion

The prognostic utility of Ki-67 in breast cancer remains a significant issue especially in low resource settings where genomic assays are not available. Ki-67 is a feasible and cheap biomarker of tumor proliferation that can be used in directing treatment and patient risk stratification(31).

In our investigation, 60.8% of the patients had high Ki-67 expression when a 14% cut off was used. This figure is considerably different from those of Indian (15.1%) and Iranian studies (28.0%) whose results are much lower than ours (32, 33). The reason for this could be the tumor biology, the demographics of the patients, or even the laboratory protocols (34, 35). Our results, however, are not far off from those of Japan (60%) and Pakistan (58.0%) where high Ki67 also indicated advanced disease and HER2 positivity (36,37).

Strong correlations were found between high Ki67 expression and ER negativity, PR negativity, HER2 positivity, higher tumor grade, advanced Pathologic stage, lymph node metastasis, and aggressive molecular subtypes including Luminal B like, HER2 enriched, and Triple negative (38-40). These are basically the same findings as in the studies from Rome, Japan, and Thailand which have demonstrated the relationships of Ki-67 with the unfavorable tumor features such as receptor status and molecular subtype(41-43).

Surprisingly, older age (60 years) patients had lower Ki67 index in the bivariate analysis, which lost it's statistical significance in the multivariable model. This is in contrast to a few studies such as those from Japan where they reported lower Ki-67 expression in older patients (44, 45). Of the three factors, HER2 positivity (adjusted odds ratio: 11.35), lymph node involvement (5.85), and molecular subtype (4.98 for non-Luminal A like) were the independent predictors of the high Ki-67 expression in the final model (42).

Such a close association has also been reported in Pakistani and Indian studies, which demonstrated that HER2 positivity and aggressive subtypes marker were highly correlated with elevated levels of Ki-67(39).

Furthermore, the 1 year follow-up results showed that patients with a high expression of Ki-67 had a higher number of recurrences, progression, and even death(32).

Keeping with the theme, looking at the studies done in England and China which confirm the ability of Ki-67 to prognosticate both disease-free survival and overall mortality, especially in ER-positive and HER2-negative tumors(40).

6.1. Strength and Limitations of the study

The study was carried out by using a standardized SPSS version 27 to ensure methodological consistency, and the one-year follow-up period made it possible to evaluate short-term clinical outcomes. The research being the first in Ethiopia, thoroughly assessing Ki-67 expression in conjunction with major clinicopathological parameters, thus, significantly adding to the local as well as global breast cancer knowledge pool.

The cutoff of 14% Ki-67 was used in the study as it is a threshold adopted in a recent international consensus guideline to separate high and low proliferation. There are different cut off points that can be use to subclassify as high and low, although such decisions that refer to clinical practice realities, may cause classification bias and hence, their results should be carefully interpreted.

Among the limitations is the retrospective, hospital-based design, limiting the extent to which findings could represent the general population.

Only short-term effects are likely to be reflected by the one-year follow-up period, and the absence of some variables, such as tumor size and detailed staging, may have led to a restricted analysis. At the same time, the study boasts its sample size, complete IHC data, and a variety of statistical models that have had enhanced the consistency of the findings.

7. Conclusion and Recommendation

7.1. Conclusion

This Research showed that 60.8% of breast cancer patients demonstrated high Ki-67 expression which reached or exceeded 14%. High Ki-67 levels showed a strong link to aggressive tumor characteristics which included ER and PR negativity and HER2 positivity and advanced Pathologic stage and higher grade and lymph node invasion and aggressive molecular subtypes which included Luminal B like and HER2-enriched and Triple-negative. The analysis identified three independent factors which tend to be associated with high Ki-67 expression: HER2 positivity and lymph node involvement and non-Luminal A like molecular subtype. The unadjusted analysis showed that older age, 60 years or older had a better outcome but this effect disappeared after statistical correction. The one-year follow-up showed that patients with high Ki-67 levels experienced more recurrence and progression and death which confirmed its value as a prognostic indicator. The research demonstrates that Ki-67 functions as an effective clinical tool which Ethiopian doctors can use to assess breast cancer patient risks and determine their treatment needs when genomic tests remain unavailable.

7.2 Recommendations

Clinical practice: Ki-67 index should be added to the standard breast cancer pathology report at Tikur Anbessa Specialized Hospital and any other centers in Ethiopia. Adding Ki-67 not only improves prognostic prediction but also helps in making treatment decisions especially in cases of hormone receptor-positive and HER2-positive patients.

In line with clinical practice, Ki-67 continues to be a valuable prognostic marker in breast cancer management. For a resource-limited setting like Ethiopia, where advanced molecular profiling is hardly accessible, Ki-67 can be a very useful surrogate marker for making therapeutic decisions and identifying patients who may benefit from more aggressive treatment strategies(39).

Policy and resource allocation: Regional health authorities need to set a top priority for ensuring that referral hospitals are equipped with the means for standardized Ki-67 immunohistochemistry tests. To maintain consistency and reproducibility of Ki-67 scoring, it is necessary to have training and quality assurance programs.

Patient management: Physicians should consider Ki-67 results with ER, PR, and HER2 statuses for a better molecular subtype classification which helps in treatment personalization. More aggressive systemic therapies and more frequent monitoring could be warranted for patients exhibiting high Ki-67 expression.

Future research: Comprehensive studies involving multiple centers and a larger number of patients, with follow-up for an extended period, would be ideal for ascertaining the validity of Ki-67 as a prognostic marker independent of other factors in the Ethiopian population.

Also, future research should investigate the most appropriate Ki-67 threshold values for the local clinical environment and determine its predictive value in treatment response.

Public health impact: The regular application of Ki-67 in breast cancer treatment could be a significant landmark in Ethiopia's efforts to enhance risk stratification, efficiently allocate treatments, and eventually lower breast cancer mortality.

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Annexes

Annex : Breast cancer Ki-67 Data Collection Checklist

Study Title: Association of Ki-67 Expression with Clinicopathological Parameters and Prognostic Significance in Breast Cancer at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia
Site: Tikur Anbessa Specialized Hospital (TASH), Addis Ababa
Type: Cross-sectional analytic study
Data Collector Name: _____
Date: ___ / ___ / _____
Patient Code (Anonymized): _____

SECTION A: PATIENT DEMOGRAPHICS

1. What is the patient's age at diagnosis?
   ☐
2. Menopausal status:
   ☐ Pre-menopausal ☐ Post-menopausal ☐ Unknown

SECTION B: TUMOR CHARACTERISTICS

3. Tumor size (in cm): _____
4. Tumor laterality:
   ☐ Left ☐ Right ☐ Bilateral ☐ Unknown
5. Pathologic stage (based on TNM/AJCC):
   ☐ Stage I ☐ Stage II ☐ Stage III ☐ Stage IV ☐ Unknown
6. Lymph node involvement:
   ☐ Positive ☐ Negative ☐ Not assessed

7. Distant metastasis at diagnosis:

☐ Yes ☐ No ☐ Not known

SECTION C: HISTOPATHOLOGY

8. Histological type:

☐ Invasive ductal carcinoma

☐ Invasive lobular carcinoma

☐ Other (specify): _____

9. Tumor grade:

☐ Grade I (well-differentiated)

☐ Grade II (moderately differentiated)

☐ Grade III (poorly differentiated)

☐ Unknown

10. Lymphovascular invasion:

☐ Present ☐ Absent ☐ Not reported

11. Perineural invasion:

☐ Present ☐ Absent ☐ Not reported

SECTION D: IMMUNOHISTOCHEMISTRY (IHC)

12. Estrogen Receptor (ER) status:

☐ Positive (% if available): _____

☐ Negative

☐ Unknown

13. Progesterone Receptor (PR) status:

☐ Positive (% if available): _____

☐ Negative

☐ Unknown

14. HER2 status (IHC or FISH if done):

☐ Positive

☐ Negative

☐ Equivocal

☐ Not tested

15. Ki-67 Proliferation Index (%): _____

SECTION E: MOLECULAR SUBTYPE (Derived from IHC)

16. Molecular subtype:

☐ Luminal A like

☐ Luminal B like

☐ HER2-enriched

☐ Triple negative

☐ Not classifiable

SECTION F: TREATMENT DATA (Optional if available)

17.Type of surgery performed:

☐ Mastectomy ☐ Breast-conserving (Lumpectomy) ☐ Core biopsy ☐ None ☐ Unknown

18.Chemotherapy received:

☐ Yes ☐ No ☐ Unknown

20.Radiotherapy received:

☐ Yes ☐ No ☐ Unknown

21 .Hormonal therapy (e.g., Tamoxifen):

☐ Yes ☐ No ☐ Unknown

SECTION G: Patient outcome

☐ **Disease-free**

☐ **Local recurrence**

☐ **Regional recurrence (lymph nodes)**

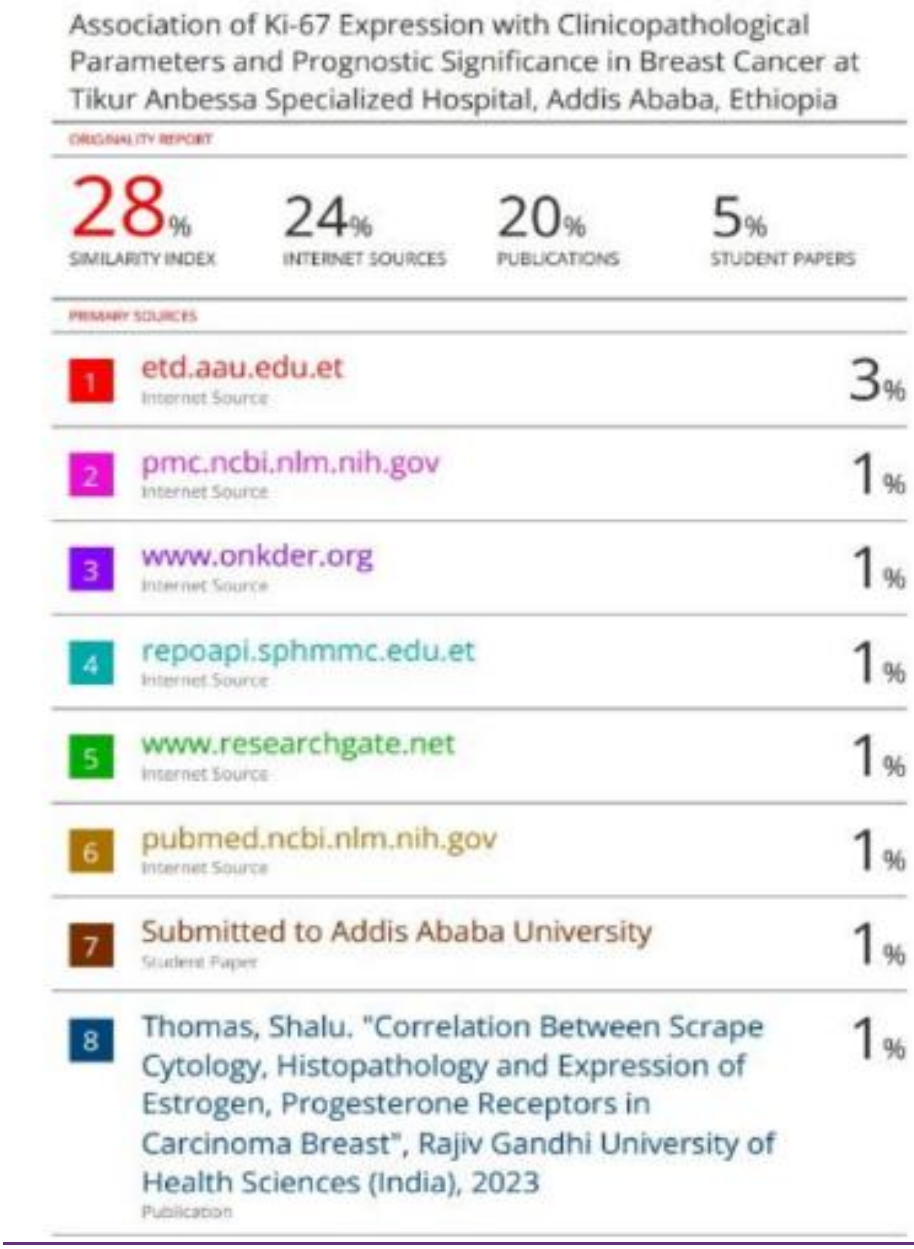
☐ **Distant recurrence / Metastatic disease**

☐ **Progressive disease**

☐ **New primary breast cancer (contralateral or ipsilateral)**

☐ **Unknown / Not assessed**

☐ **Died**



Association of Ki-67 Expression with Clinicopathological Parameters and Prognostic Significance in Breast Cancer at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

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