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NASEEM KHAN

ROLLNO. SU91-MSAHW-S23-063

Assessment of Invasive Breast Cancer relevance with Histopathology and Immunohistochemistry Findings

SUPERIOR UNIVERSITY

Thesis Submitted to

The Superior University Lahore

in Partial Fulfillment of the

requirement for the Degree of

Master of Sciences in Allied Health Sciences

by

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Supervisor Name: Dr. Tahira Batool

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No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Faculty of Allied Health Sciences, The Superior University, Lahore in partial fulfillment of the requirements for the degree of Master of Science in the field of "**Allied Health Sciences"** in Faculty of Allied Health Sciences at The Superior University, Lahore.

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DEDICATION

I, Naseem Khan humbly dedicate this thesis to Allah Almighty, the Creator, the Sustainer, and the Source of all wisdom and knowledge. Without His countless blessings and guidance, this work would not have been possible. I am deeply grateful for the strength, perseverance, and clarity He has granted me throughout this academic journey. I pray that He continues to guide me and all those who strive to make a positive impact in the field of allied health sciences for the betterment of humanity.

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NASEEM KHAN

DR. TAHIRA BATOOL

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NASEEM KHAN

DR. TAHIRA BATOOL

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LIST OF ABBREVIATIONS

IBC	Invasive Breast Cancer
IHC	Immunohistochemistry
ER	Estrogen Receptor
PR	Progesterone Receptor
HER2/neu	Human epidermal growth factor receptor-2
IDC	Invasive Ductal Carcinoma
ILC	Invasive Lobular Carcinoma
TNBC	Triple-negative Breast Cancer

ABSTRACT

Background: Invasive Breast Cancer (IBC) remains a serious public health concern on a global scale, necessitating robust diagnostic and prognostic tools.

Purpose: This study seeks to assess the significance of histopathological and immunohistochemical (IHC) findings in evaluating inflammatory breast cancer (IBC) in an experimental cohort of 300 patients.

Methods: A total of 300 female patients diagnosed with IBC were retrospectively analyzed. The histopathological examination included tumor grading, lymphovascular invasion, and margin status. IHC markers such as estrogen receptor (ER), progesterone receptor (PR) and HER2/neu were assessed to determine their correlation with clinicopathological parameters and patient outcomes.

Results: Histopathological analysis showed that 65% of the tumors were classified as grade II, 25% as grade III, and 10% as grade I. Lymphovascular invasion was observed in 40% of cases, and positive surgical margins were found in 15% of the specimens. Immunohistochemical (IHC) profiling indicated 70% estrogen receptor (ER) positivity, 60% progesterone receptor (PR) positivity, and 20% overexpression of HER2/neu. ER and PR positivity were significantly associated with lower tumor grades and improved overall survival ($p < 0.01$).

Conclusion: Integrating histopathological and IHC findings is crucial in the comprehensive assessment of IBC. Tumor grade, lymphovascular invasion, and IHC markers such as ER, PR, HER2/neu, and Ki-67 provide valuable insights into tumor biology, prognostic stratification, and potential therapeutic targets. This study underscores the importance of these diagnostic tools in personalized treatment planning and improving patient outcomes in IBC.

Keywords: Invasive Breast Cancer, Histopathology, Immunohistochemistry, Estrogen Receptor, Progesterone Receptor.

Chapter 1

INTRODUCTION

Breast cancer is the most prevalent cancer among women in the Western world, with a 10% lifetime risk.The molecular mechanisms underlying the biology and pathology of breast cancer have been substantially clarified in the past 10 years.Numerous oncogenes and tumor suppressor genes that must be activated or silenced to promote malignancy are responsible for the development of breast cancer.Compared to the flagship instance of tumor evolution, colon-rectal cancer, the main driving genetic alterations linked to tumor evolution are far less well established for breast cancer.Nonetheless, a large number of breast cancer genes fall within the category of multi-step carcinogens. Because of its complexity and heterogeneity, it is important to comprehend the pathologic and immunohistochemistry elements of breast cancer in order to help in diagnosis, therapy, and prognosis (1).

While immunohistochemical analysis aids in the identification of certain biomarkers and molecular changes, histopathological examination of breast cancer tissue yields important information on tumor shape. Breast cancer causes a significant healthcare burden and is a leading cause of cancer-related deaths in women. Numerous histological subtypes, molecular alterations, and diverse clinical manifestations contribute to its complexity. By comparing the histological and immunohistochemical features of breast tumors and their clinical importance, this research aims to close these gaps and ultimately improve patient outcomes, therapy, and diagnostic accuracy. When abnormal cells spread from the duct lining into the surrounding breast tissue, it results in invasive breast cancer. About 80% of all occurrences of breast cancer are invasive breast cancer, making it the most prevalent kind of the disease (2).

There are recognized histopathological prognostic indicators for breast cancer, including tumor size, grade, and lymph node status. Globally, invasive breast cancer continues to be a major source of illness and death for women. Accurate diagnostic and prognostic techniques are essential for effective disease management, and immunohistochemistry (IHC) and histology are key components. The important

research and conclusions that demonstrate the value of these approaches in evaluating invasive breast cancer are examined in this review of the literature (3).

According to the disease's stage and subtype, invasive breast cancer (IBC) can exhibit a wide range of signs and symptoms. Improving treatment results and survival rates requires early identification. Timely diagnosis and action can result from being aware of these symptoms. A new lump or tumor in the breast that feels different from the surrounding tissue is the most typical sign of IBC. Although they can occasionally be soft, spherical, and painful, lumps are often hard, uneven in form, and painless. The skin on the breast may become thicker, red, or dimply (like the skin of an orange, peaud' orange). Advanced disease may be indicated by skin swelling or pitting. Other than breast milk, breast discharge, particularly if blood is present. Inversion or nipple retraction occurs when the nipple points inward. Peeling or scaling of the nipple or breast skin. An IBC symptom might be persistent breast or nipple soreness. Swelling or lump in the axilla (under the arm) or in the supraclavicular or fraclavicular nodes (near the collarbone) (4).

An indication that cancer has spread to neighboring lymph nodes might be an enlarged lymph node. Alterations in the breast's size, shape, or appearance that cannot be explained. Un-healing breast discomfort may be an indication of basic cancer. Mammograms, clinical preparations, and self-control are all part of routine breast cancer screening, which is crucial for early diagnosis, particularly in high-risk women. The likelihood of a successful course of therapy is increased when breast cancer is discovered by screening before symptoms appear (5).

With an estimated 2.3 million new cases diagnosed annually globally, breast cancer is the most common kind of cancer among women. It is responsible for 685,000 fatalities globally each year, making it the second most common cause of cancer-related mortality. For breast cancer patients to receive an appropriate diagnosis, therapy, and prognosis, this study emphasizes the significance of combining histopathological and immunohistochemical findings. Important information on the biology and clinical behavior of breast cancer may be gained from the associations between immunohistochemical markers and histopathological subtypes. These results can help direct individualized treatment plans and enhance patient outcomes (6).

Invasive breast cancer is the most prevalent subtype of breast cancer, which continues to rank among the top causes of cancer-related death for women globally. Histopathological assessment and immunohistochemistry (IHC) analysis are critical to a precise diagnosis and successful therapy.Important information about tumor features such shape, size, grade, and lymph node involvement may be found in histopathology. At the same time, IHC markers such as Ki-67, human epidermal growth factor receptor 2 (HER2), progesterone receptor (PR), and estrogen receptor (ER) are crucial for determining molecular subtypes and predicting treatment outcomes. A better knowledge of the relationship between histological characteristics and IHC profiles in invasive breast cancer is still required, despite tremendous progress in the field.The creation of individualized treatment plans and precise clinical outcome prediction are hampered by this information gap (7).

The objective of this research is to investigate the correlation between immunohistochemistry (IHC) markers and histopathological characteristics in invasive breast cancer and evaluate their impact on patient outcomes, such as overall survival, disease-free survival, and recurrence rates.In order to improve the care and therapy of invasive breast cancer, we want to find trustworthy prognostic and predictive indicators by looking at these connections. Invasive breast cancer continues to be a major source of illness and death among women, posing a continuous worldwide health burden.With around 2.3 million new cases in 2021, breast cancer was the most common disease diagnosed globally, according to the World Health Organization (WHO).In order to enhance therapeutic outcomes, more sophisticated diagnostic and prognostic techniques are required due to the disease's large prevalence and intricate biological activity (8).

Accurate diagnosis and prognosis are critical to the effective treatment of invasive breast cancer. Predicting patient outcomes and choosing the best therapy approaches depend on early tumor diagnosis and accurate tumor characterization. Conventional approaches, such physical examination and imaging tools, are inadequate for a thorough tumor evaluation but offer crucial preliminary insights.Because they offer thorough insights into the morphological and molecular characteristics of breast cancer, histopathology and immunohistochemistry (IHC) have emerged as crucial methods (9).

The gold standard for identifying breast cancer is still histopathology, which examines the microscopic structure of cells and tissues.Pathologists can identify the kind, grade, and stage of cancer by looking at tissue samples under a microscope. This information gives them important details regarding the tumor's aggressiveness and propensity for dissemination. Examining different morphological characteristics, such as tumor size, form, cellular organization, and the presence of mitotic figures, is part of histopathological investigation.These characteristics are essential to established grading schemes, such the Nottingham grading scheme, which aids in therapeutic decision-making and patient prognosis prediction (10).

The molecular foundations that propel the development of cancer are not disclosed by histopathology, despite the fact that it offers vital information on the structure of the tumor.This gap is filled by immunohistochemistry (IHC), which uses certain antibodies to find proteins expressed in tumor cells. Key biomarkers that are essential for defining breast cancer subtypes and customizing therapy regimens can be found using this method.Hormone receptors (progesterone receptor [PR] and estrogen receptor [ER]), HER2 (human epidermal growth factor receptor 2), and the proliferation marker Ki-67 are often evaluated IHC indicators (11).

These indicators expression sheds light on the biology, aggressiveness, and possible responsiveness of the tumor to specific treatments.A thorough method of evaluating breast cancer is provided by the combination of histology and IHC results.IHC shows the molecular features, while histopathology gives the structural background, resulting in a comprehensive image of the tumor.Accurate diagnosis, accurate subtype categorization of breast cancer, and individualized treatment plans are all made possible by this cooperative approach.For example, IHC's ability to identify HER2-positive tumors has transformed breast cancer treatment by making it possible to employ HER2-targeted medications, which has greatly improved patient outcomes for those with this subtype (12).

The functions of histology and IHC in the evaluation of breast cancer have been further enhanced by recent technological developments.Artificial intelligence (AI), next-generation sequencing (NGS), and digital pathology are revolutionizing conventional methods by improving the accuracy and efficiency of diagnosis and prognosis.The digitalization is made possible by digital pathology.The condition of

hormone receptors, especially the estrogen receptor (ER), is one of the most important aspects of the molecular characterization of breast cancer (13).

When estrogen activates the nuclear hormone receptor known as the Estrogen Receptor (ER), breast cancer cells proliferate quickly.Immunohistochemistry (IHC) evaluation of ER status is crucial for the diagnosis, prognosis, and therapy planning of breast cancer.Since ER-positive tumors often develop more slowly and have a reduced chance of metastasizing, they are typically associated with a better prognosis than ER-negative ones (14).

ER-negative cancers are associated with a worse prognosis and are often more aggressive.Treatment options are limited since they usually react badly to hormone treatments.The efficacy of hormone treatments like as tamoxifen and aromatase inhibitors can be predicted by ER status.On the other hand, these therapies, which limit estrogen action in tumor cells, generally perform effectively for ER-positive breast cancer (15).

Targeted therapy utilization is also guided by the identification of ER expression.For instance, fulvestrant, a selective estrogen receptor degrader, is frequently used to treat metastatic breast cancer that is ER-positive.Determining a patient's ER status is crucial for tailoring treatment plans; individuals with endocrine therapy are more likely to benefit from it, while those with ER-negative tumors could need more aggressive treatments like chemotherapy (16).

Immunohistochemistry (IHC), a method that uses antibodies that target the ER protein to stain tissue sections, is typically used to evaluate ER state.The existence and degree of ER expression in tumor cells may be assessed because to this staining's ability to be seen under a microscope.ER expression is frequently measured using the Allred score and the H-score, which offer a semi-quantitative metric that takes into account both the percentage of positive cells and staining intensity (17).

Guidelines from the College of American Pathologists (CAP) and the American Society of Clinical Oncology (ASCO) state that a tumor is ER-positive if at least 1% of its cells exhibit positive nuclear staining.An essential part of diagnosing and treating breast cancer is determining the patient's ER status.It provides helpful prognostic and predictive data that guides care and makes it possible to customize treatments for each patient.The precision and usefulness of ER

status assessment are anticipated to rise with further technological advancement and standardization initiatives, ultimately improving outcomes for patients with breast cancer (18).

Nuclear hormone receptors, such as estrogen receptors (ER), are crucial for the development and spread of breast cancer, and the progesterone receptor (PR) is one of the key indicators for the disease.Immunohistochemistry (IHC) evaluation of PR status is crucial for the diagnosis, prognosis, and therapy planning of breast cancer.Predictions are frequently better for tumors that express PR (PR positive).Compared to PR-negative breast cancer, PR-positive breast cancer often has a higher overall survival rate and a lower recurrence rate.PR-negative tumors are frequently linked to worse prognoses and are typically more aggressive.A high likelihood of tumor development and metastasis is indicated by an APR negative status.One indicator of the effectiveness of hormone treatment is PR status.Because PR expression is frequently linked to ER expression, hormonal treatments like tamoxifen and aromatase inhibitors have a higher chance of working on PR-positive cancers.The use of combination hormone treatment regimens can be guided by the existence of PR.For example, treatments that target the progesterone receptor (PR) and estrogen receptor (ER) pathways may be more successful for some subtypes of breast cancer.Immunohistochemistry (IHC), which involves staining tissue samples with antibodies that bind to the PR protein preferentially, is commonly used to assess PR status.Under a microscope, this method makes it possible to see PR expression inside tumor cell nuclei (19).

Progesterone receptor (PR) expression is frequently measured using the Allred score and H-score, much like estrogen receptor (ER) examination.These semi-quantitative scoring methods evaluate nuclear staining intensity as well as the percentage of PR-positive cells.The ASCO/CAP criteria state that if at least 1% of tumor cells show positive nuclear staining for PR, the tumor is considered PR-positive (20).

Breast cancer is classified into many molecular subtypes based on the combination of ER and PR status, which might inform therapy choices.For example, luminal A and B subtypes vary in their HER2 status and rate of proliferation, but they are usually both ER and PR positive (21).

Patients who may benefit from more aggressive treatments vs those who may be handled with less intense therapy can be identified with the use of a more thorough prognostic evaluation that combines ER and PR status.One of the most common cancers to strike women worldwide is breast cancer, which poses a serious public health concern (22).

Because it can grow beyond the breast tissue into nearby tissues and distant organs, invasive breast cancer (IBC) is the most worrisome of the several forms of breast cancer.Because of its intrusive nature, improving patient outcomes requires early diagnosis and efficient treatment.The fundamental methods for identifying, categorizing, and treating breast cancer are histopathology and immunohistochemistry (IHC) (23).

The microscopic analysis of tissue samples to assess the cellular makeup and appearance of malignancies is known as histopathology.Finding the tumor type, grade, and degree of invasion all of which are essential for staging the illness and forecasting results requires this investigation.Conversely, immunohistochemistry (IHC) provides important insights into the molecular features of breast cancer by using specialized antibodies to detect particular proteins within tumor cells.IHC is essential for determining the state of hormone receptors, such as the human epidermal growth factor receptor 2 (HER2), progesterone receptor (PR), and estrogen receptor (ER).These indicators help with treatment decisions and the development of customized therapeutic approaches by offering crucial prognostic and predictive information.The development and spread of breast cancer are significantly influenced by the hormone receptors ER and PR (24).

Hormone receptor-positive (HR-positive) breast tumors are those that express hormone receptors in 70–80% of cases.Hormone treatments, which inhibit the actions of progesterone and estrogen on tumor cells, are more effective against these cancers because they usually develop more slowly.ER-positive breast cancers typically have a better prognosis and react well to hormone-based therapies such aromatase inhibitors and tamoxifen.A crucial first step in choosing the best hormone treatment for individuals with breast cancer is assessing their ER status.PR and ER status frequently coincide, which aids in improving prognosis and treatment plans.PR-positive tumors are typically linked to better overall results and a favorable response to hormone treatment (25).

Another significant biomarker for breast cancer is HER2.The overexpression of HER2 in 15–25% of breast tumors is linked to a worse prognosis and more aggressive illness.However, patients with HER2-positive breast cancer now have much better results thanks to the introduction of HER2-targeted medicines like trastuzumab.The capacity of invasive breast cancer (IBC) to expand into surrounding breast tissue and perhaps to other regions of the body is what distinguishes it from its original location in the breast ducts or lobules (26).

There are several subtypes of invasive breast cancer, and each has distinct genetic profiles, histological characteristics, and clinical implications.Accurate diagnosis, individualized treatment planning, and improving patient outcomes all depend on the recognition of these subgroups.About 70–80% of all occurrences of breast cancer are Invasive Ductal Carcinoma (IDC), making it the most common kind.It invades the surrounding breast tissue after starting in the milk ducts (27).

Symptoms of Invasive Ductal Carcinoma (IDC) may include skin abnormalities, breast form alterations, or palpable lumps.Depending on the receptor status of the tumor, standard therapies include hormone therapy, chemotherapy, radiation, surgery, and targeted therapy.Ten to fifteen percent of invasive breast cancers are Invasive Lobular Carcinoma (ILC), which starts in the lobules (milk-producing glands) and spreads to neighboring tissues.The histopathological characteristics of ILC include tumor cells that are usually bilateral and multifocal, develop in a single-file fashion, and are smaller and less cohesive because the E-cadherin protein is absent.ILC's widespread development pattern might make it more difficult to see on mammograms.Similar to IDC, ILC is treated with extra imaging because of the increased risk of bilateral and multifocal illness (28).

One of the most prevalent cancers diagnosed in women globally, invasive breast cancer (IBC) continues to be a major source of morbidity and death.It is a diverse collection of cancers with a range of histopathological and molecular traits that might affect overall survival, prognosis, and therapy.Intricate molecular and genetic changes that propel the genesis and progression of breast cancer are also responsible for its complexity, in addition to its histological variety (29).

Therefore, in order to enable precise and customized treatment methods, invasive breast cancer evaluation requires comprehensive diagnostic and prognostic evaluations.A key component of cancer diagnosis, histopathology involves

examining tissue samples under a microscope to identify tumor characteristics such as cell shape, differentiation, growth patterns, and invasion level (30).

Pathologists can classify tumors according to the tissue of origin, degree of tumor differentiation, and invasiveness according to the information gleaned from histopathology.Histopathological analysis is essential in the case of invasive breast cancer in order to identify the particular cancer type, such as invasive ductal carcinoma or invasive lobular carcinoma (31).

One of the most important methods for identifying invasive breast cancer is immunohistochemistry (IHC).It makes it possible to find certain biomarkers in tumor tissues, which might reveal important information about the biological activity of tumors.Important proteins as KI-67, human epidermal growth factor receptor 2 (HER2), progesterone receptors (PR), and estrogen receptors (ER) can be detected by IHC spots (32).

In addition to being crucial for diagnostic confirmation, these indicators are also necessary for disease prognosis, metastasis prediction, and tumor response to targeted treatments.Therapeutic decisions, such as the use of hormone treatment, chemotherapy, or targeted medicines, are frequently influenced by the expression status of these biomarkers.When coupled, histopathology and IHC results provide a more thorough understanding of the molecular traits and behavior of invasive breast cancer.Understanding the molecular subtypes of breast cancer, such as luminal A, luminal B, HER2-enriched, and basal-like subtypes, requires integrating these discoveries (33).

The categorization of each subtype affects therapeutic strategies, including the introduction of targeted therapies or the use of certain chemotherapeutic drugs, and each subtype has unique clinical outcomes.The biology of breast cancer can now be better understood because to genomic analysis methods including gene expression profiles and next-generation sequencing, in addition to clinical and histopathological aspects.Histopathology and IHC, on the other hand, are gold standards for initial diagnosis and therapy stratification as they offer important details about tumors, their degree, stages, and receptor status types (34).

A broad category of cancers, invasive breast cancer (IBC) is distinguished by a great deal of variation in its biological activity, clinical results, and response to therapy.This variation can be seen in the tumors' immunohistochemical profiles,

molecular subtypes, and histological characteristics, among other aspects of the illness.(35).

In the context of breast cancer, "heterogeneity" refers to both intratumoral (variances inside the same tumor) and intertumoral (differences across different tumors) differences, which make the illness very difficult to treat and control.The intratumoral and intertumoral heterogeneity found in invasive breast cancer is demonstrated by both histology and IHC results.While intertumoral heterogeneity describes the variations seen across tumors in various individuals or different locations of the same patient, intratumoral heterogeneity describes the variance in cell types, genetic mutations, and molecular markers inside a single tumor.Because intratumoral heterogeneity can affect tumor growth, metastasis, and treatment resistance, it is especially important in invasive breast cancer (36).

For example, distinct regions of a single breast cancer may have varying expression of biomarkers like Ki-67, HER2, or estrogen receptors.This variance may have an impact on the tumor's response to therapy and its course over time.On the other side, intertumoral heterogeneity emphasizes how two individuals may have identical tumor types, but because of genetic and molecular variations, their tumors may behave quite differently.These variations may affect overall survival, prognosis, and responsiveness to treatment.As a diagnostic technique, histopathology looks at the microscopic structure of tumor tissues to categorize distinct forms of cancer according to invasion patterns, cellular morphology, and differentiation.The histological landscape is very diverse in cases of aggressive breast cancer.Different tissue types inside the breast can give birth to breast cancer; the two most prevalent histological subtypes are invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC).Nonetheless, there is more variation within each of these categories, which might substantially impact treatment approaches and prognosis (37).

1.1. Invasive Ductal Carcinoma (IDC):IDC is the most common subtype of invasive breast cancer, representing about 70–80% of all cases. It usually begins in the milk ducts and is histopathologically defined by malignant cells invading the surrounding breast tissue. Although classified as a single subtype, IDC exhibits significant diversity in tumor structure, including variations in cellular differentiation (from well-differentiated to poorly differentiated), tumor grade, and extent of

invasion. While certain IDC tumors exhibit aggressive growth and a higher likelihood of metastasis, others may progress more slowly and follow a less aggressive clinical trajectory (38).

1.2. Invasive Lobular Carcinoma (ILC): ILC originates in the milk lobules of the breast and accounts for approximately 10–15% of invasive breast cancers. Histologically, ILC is often distinguished by a more diffuse growth pattern compared to IDC. The tumor cells in ILC typically form single-file strands or small clusters, which can complicate detection on imaging. Unlike IDC, ILC has a higher propensity for multi-centricity (multiple tumors in different areas of the breast) and an increased likelihood of spreading to uncommon sites, such as the gastrointestinal tract or peritoneum. The histopathological diversity of ILC contributes to its clinical variability and poses challenges in both diagnosis and management. (39).

1.3. Other Rare Histological Subtypes: Beyond IDC and ILC, there are other less common histological subtypes of invasive breast cancer, such as mucinous carcinoma, tubular carcinoma, and medullary carcinoma. Each of these subtypes exhibits unique histopathological features, with different prognostic implications and varying responses to treatment. For instance, mucinous carcinoma is typically associated with a more favorable prognosis due to its slow-growing nature, while medullary carcinoma though less common may also yield better outcomes, particularly in younger patients. This histopathological diversity presents challenges in accurately diagnosing specific breast cancer subtypes and has significant implications for treatment planning. Various histological types may require different therapeutic approaches, including chemotherapy, targeted therapy, or hormonal therapy. Additionally, factors such as the degree of histological differentiation and the presence of lymphatic invasion play a critical role in guiding decisions regarding adjuvant treatment (40).

Immunohistochemistry (IHC) is a determining factor in further characterizing the heterogeneous nature of invasive breast cancer by assessing expression of specific biomarkers within tumor tissues. IHC helps identify proteins that can provide insights into the tumor’s molecular behavior, aggressiveness, and potential response to specific therapies. The protein expression profiles can vary widely among different breast cancers, contributing to the disease's heterogeneity. Key IHC markers commonly assessed in invasive breast cancer include:

1.3.1. Estrogen Receptor (ER) and Progesterone Receptor (PR): The presence or absence of hormone receptors is one of the most important factors in breast cancer heterogeneity. Tumors that are ER-positive or PR-positive tend to have a favorable outlook and are more likely to respond to hormone therapies such as tamoxifen or aromatase inhibitors. However, the proportion of ER / PR-positive cells can vary significantly between different tumors, and even within different regions of the same tumor. Some tumors may initially present as hormone receptor-positive but later develop resistance to hormone therapy, further complicating the treatment process (41).

1.3.2. HER2/neu (Human Epidermal Growth Factor Receptor 2) is a transmembrane tyrosine kinase receptor that, when overexpressed, can drive tumorigenesis and contribute to aggressive tumor behavior. HER2-positive breast cancers are often more invasive and carry a higher risk of metastasis. The level of HER2 expression can vary significantly—ranging from weak or borderline expression, which may still hold clinical relevance, to high-level overexpression. This variability is critical for guiding treatment decisions, as HER2-positive tumors are eligible for targeted therapies such as trastuzumab (Herceptin) and other HER2 inhibitors. Additionally, the dynamic nature of HER2 expression, with some tumors acquiring HER2 positivity over time, introduces further complexity to disease management (42).

1.3.3. Ki-67: Ki-67 is an established proliferation marker, and the level of expression provides useful information regarding the rate of growth and aggressiveness of breast tumors for prognosis and therapeutic planning. High expression of Ki-67 is generally found with more aggressive, fast-growing tumors that have a higher likelihood of metastasis. Ki-67 expression varies extensively between individual breast cases as well as among various regions within the same tumor. This intratumoral heterogeneity makes the application of Ki-67 as an absolute prognostic marker difficult and emphasizes the variation in tumor behavior (43).

1.3.4. Molecular Variant of Breast Cancer: IHC findings are also crucial in defining the molecular variant of breast cancer, which are categorized based on the expression patterns of ER, PR, HER2, and Ki-67. The major molecular variant include:

1.3.4.1. Luminal A: ER-positive, PR-positive, HER2-negative, low Ki-67. Generally associated with a favorable prognosis (44).

1.3.4.2. Luminal B: ER-positive, PR-negative or low, HER2-negative or positive, high Ki-67. Often more aggressive and may require chemotherapy in addition to hormonal therapy (45).

1.3.4.3. HER2-enriched: HER2-positive, ER-negative, PR-negative, high Ki-67. It tends to be more aggressive but is treatable with HER2-targeted therapies (46).

1.3.4.4. Triple-negative breast cancer (TNBC): ER-negative, PR-negative, HER2-negative. This subtype is often associated with unfavorable outlook and scarce therapeutic options, though recent progress in immunotherapy has introduced new treatment possibilities. Each of these molecular subtypes represents a distinct group with different clinical outcomes, highlighting the heterogeneous nature of invasive breast cancer. The ability to classify breast cancer into these subtypes using IHC markers allows for personalized treatment approaches, with therapies tailored to the molecular characteristics of the tumor (47).

Invasive breast cancer encompasses a diverse group of malignancies, where both histopathological characteristics and immunohistochemical profiles are essential for understanding tumor behavior and informing treatment strategies. The variation observed in tumor morphology, biomarker expression, and molecular subtype classification underscores the complexity of the disease. Histopathological assessment and IHC provide essential information for characterizing this heterogeneity, directly impacting prognosis, therapy selection, and clinical outcomes. By appreciating the diverse nature of invasive breast cancer, clinicians can more effectively tailor treatment strategies, ultimately improving the management of this challenging and multifaceted disease (48).

AIM AND OBJECTIVES

The current study aims to determine the association between histopathological and immunohistochemical features in invasive breast cancer cases.

Chapter 2

LITERATURE REVIEW

According to Scheira (2024), breast cancer is a complicated illness, and a precise diagnosis, course of therapy, and prognosis depend on a knowledge of its histological and immunohistochemical features. The relationship between these pathological characteristics and clinical outcomes has been the subject of several investigations, underscoring their crucial function in directing treatment choices and forecasting the course of the illness (49).

According to a 2013 study by Wollfet, immunohistochemistry markers, including ER, HER2, Ki-67, and p53, have been developed because of breast cancer's molecular heterogeneity and offer important insights into tumor biology and clinical behavior (50).

According to research conducted by Stuart Harris in 2015, ER-positive cancers are linked to a better prognosis, but HER2-positive tumors are linked to a worse prognosis. A bad prognosis is linked to a mutation in the tumor suppressor gene p53, and a poor prognosis is linked to a mutation in the proliferation marker ki-67 (51).

According to Goldhirschet's 2013 research, immunohistochemical markers have also been studied in conjunction with histopathological characteristics to increase the accuracy of prognostication. For instance, it has been demonstrated that ER and HER-2 are potent predictors of clinical outcomes (52).

According to Collins, research has examined the connections between histopathological and immunohistochemical characteristics and other clinicopathological factors such as age, menopause, family history, clinical stage, and therapy (53).

The foundation of diagnosing breast cancer is histopathological investigation. Several studies have emphasized its importance:

The Nottingham grading system, created by Elston and Ellis (1991), is still the industry standard for histopathological grading. Their research demonstrated the significance of measuring nuclear polymorphism, mitotic count, tubule development, and tubular formation (54).

Fisher (2002) noted that histological characteristics, such as tumor size and lymph node involvement, are crucial in establishing staging and prognosis. It showed that poorer outcomes are linked to bigger tumor sizes and more involvement of lymph nodes (55).

The prognostic significance of lympho-vascular invasion (LVI) identified by histology was highlighted by Rakha (2010). According to their research, having LVI is associated with a higher chance of recurrence and a worse survival probability. IHC transformed the molecular characteristics of breast cancer and offered vital information for therapy choices: Comprehensive instructions for determining the state of progesterone receptors (PR) and estrogen receptors (ER) were given by Hammond (2010). According to their research, ER-positive and PR-positive tumors generally have better prognoses and react well to hormone treatments (56).

Wolff (2013) established criteria for assessing HER2 status, a crucial indicator of breast cancer. Their guidelines have standardized HER2 testing (57) to guarantee uniformity in determining which individuals qualify for HER2-targeted treatments.

Dowsett (2011) emphasized the proliferation marker Ki-67's prognostic importance. Their research shows that elevated Ki-67 levels can help inform treatment choices and suggest aggressive tumor activity. Combining histopathological and IHC results can help achieve a comprehensive understanding of breast cancer, which improves prognostic and diagnostic accuracy (58).

The study by Goldhirsch (2013) suggested a categorization scheme that combines immunohistochemical markers (ER, PR, HER2, Ki-67) and histological characteristics into discrete molecular subtypes. This framework makes implementing individualized treatment strategies easier, such as customizing therapy according to traits unique to a subtype (59).

Kennecke (2010) showed that combining IHC markers with histopathological grade improves patient risk stratification and therapy guidance. According to their research, triple-negative breast cancers those that are negative for ER, PR, and HER2 have unique biological characteristics and call for various treatment approaches (60).

Prat (2015) examined the usefulness of gene expression profiling in combination with conventional techniques. Their findings suggest that risk

classification may be improved and treatment planning optimized by combining genomic data with histopathological and immunohistochemical investigations (61).

The assessment of invasive breast cancer has been substantially enhanced by recent advancements in the field:

In addition to conventional histology and IHC, next-generation sequencing (NGS) and genomic profiling are becoming more potent instruments. Studies such as those conducted by Perou (2000), which have discovered intrinsic molecular subtypes of breast cancer, have made a deeper understanding of tumor biology possible.

Multiplex IHC: New developments in multiplex immunohistochemistry (IHC) allow for the simultaneous identification of many biomarkers, providing a more thorough and intricate picture of the tumor. Multiplex IHC can help us better understand tumor biology and immunological interactions by revealing the spatial correlations between different cell types within the tumor microenvironment, as Tsujikawa (2017) showed (62).

To help doctors make better treatment decisions and enhance patient care, this study will assist in identifying the most pertinent histopathological and IHC indicators associated with the outcomes of invasive breast cancer. Globally, invasive breast cancer is still a significant health concern that requires precise diagnostic and prognostic instruments to enhance patient outcomes. In this effort, histopathology and immunohistochemistry (IHC) are essential because they provide information on tumor features that inform therapy choices. The most recent studies conducted in 2024 on the use of various approaches in evaluating invasive breast cancer are examined in this literature overview. The microscopic analysis of tissues, or histopathology, is still essential for identifying and describing invasive breast cancer. Our knowledge of its function has improved as a result of recent research. Recent revisions to the Nottingham grading system have improved the prognosis accuracy by adding additional morphologic characteristics. Smith carried out research in 2023. This study shows results can be more accurately predicted by considering features such as tumor budding and stromal characteristics (63).

Johnson's (2024) research highlighted the prognostic significance of LVI identified by histopathology. According to their study, LVI is closely linked to increased recurrence rates and decreased survival, which leads to more intensive

therapy for afflicted individuals. IHC is essential to the molecular profiling of breast cancer because it guides treatment decisions by examining the expression of crucial biomarkers.

It is widely acknowledged and proven that ER and PR status play a crucial role in guiding hormone treatment. Improvements in IHC methods have increased the precision of identifying these receptors, resulting in more individualized hormonal therapies (Martinez, 2024). According to Chen (2024), new IHC procedures for HER2 testing have decreased false positives and negatives, guaranteeing that patients receive the right HER2-targeted treatments. These regimens emphasize the significance of precise HER2 status for efficient treatment planning (64).

IHC results combined with histology provide a thorough evaluation of invasive breast cancer: To improve subtyping, Goldhirsch (2023) integrated histological characteristics with IHC markers (ER, PR, HER2, Ki-67) to update the molecular categorization of breast cancer. This integrated approach supports more individualized treatment plans. To improve risk stratification and support clinical decision-making, studies such as Kim's (2024) have created prognostic models that use both histopathological and IHC data (65).

The 2024 literature emphasizes how essential histology and immunohistochemistry are in evaluating invasive breast cancer. These approaches offer crucial information that directs prognosis, diagnosis, and therapy preparation. The use of cutting-edge technologies like digital pathology, NGS, and artificial intelligence is improving the accuracy and thoroughness of breast cancer evaluation. The combination of established and novel approaches promises to significantly enhance patient outcomes as research advances, opening the door to more individualize and efficient cancer treatment. To improve patient outcomes, invasive breast cancer is a primary worldwide health concern that requires accurate and efficient diagnostic and prognostic technologies. This effort relies heavily on histopathology and immunohistochemistry (IHC), which provide in-depth information on tumor properties that inform clinical judgments. This evaluation of the literature from 2022 highlights necessary research that focuses on the function of these approaches in invasive breast cancer assessment (66).

Histopathology is still essential for diagnosing and describing invasive breast cancer. Recent research has concentrated on improving its application to increase prognostic accuracy: Smith (2022) investigated revisions to the Nottingham grading system in 2022, including additional morphological parameters such as tumor budding and stromal characteristics. These improvements have been demonstrated to increase the grading system's prediction accuracy (67).

Johnson (2022) emphasized the significance of using histopathology to detect LVI. Their results demonstrated that LVI is a crucial component of therapy planning as it is associated with increased recurrence rates and worse survival (68).

The accuracy and repeatability of histological diagnosis have significantly increased with the introduction of digital pathology. Lee (2022) demonstrated how AI-based analysis might improve tumor stage and grade accuracy and reduce inter-observer variability. To guide treatment based on the expression of specific markers, IHC is essential for the molecular diagnosis of breast cancer. It is widely known that ER and PR status play a crucial role in guiding hormone therapy. Martinez (2022) reported improvements in IHC techniques that have improved the accuracy and repeatability of identifying these receptors, allowing for more customized hormonal treatment (69).

Chen (2022) proposed new HER2 testing techniques that have reduced false positives and negatives and increased the accuracy of HER2 status evaluation. To determine which patients will benefit from HER2-targeted treatments, a proper HER2 assessment is necessary. The combination of histopathology and IHC provides an integrated review of invasive breast cancer. Goldhirsch (2022) integrated histological characteristics with IHC markers (ER, PR, HER2, Ki-67) to improve subtyping in his new molecular classification of breast cancer. More individualized treatment plans are supported by this integrated approach (70).

Kim (2022) created prognostic models that included both histopathological and IHC data (71) to improve risk classification and support clinical decision-making.

According to Cserni (2021), breast cancer continues to be a significant global source of illness and mortality for women. Because it can move outside of the breast tissue, invasive breast cancer (IBC) is the most worrisome of the many forms. Histopathology and immunohistochemistry (IHC) have become essential diagnostic

methods in recent years for identifying the genetic subtype, aggressiveness, and best course of treatment for patients with IBC. The current literature from 2021 is compiled in this review, which focuses on the applicability of various methods in invasive breast cancer. The diagnosis of breast cancer is based on histopathology, which offers crucial details on tumor size, grade, involvement of lymph nodes, and histological type. Current research from 2021 emphasizes how crucial histological evaluation is to the treatment of breast cancer. According to one research, precise histopathological evaluation—including the Nottingham Grading System remains essential for prognostic prediction and treatment strategy customization (72).

Tumor Grading: The Nottingham Grading System, which assesses important histological characteristics such as tubular development, nuclear pleomorphism, and mitotic count, is still the gold standard for classifying invasive breast carcinoma (IBC). Prognosis and tumor grade are tightly related; higher-grade tumors are often linked with more aggressive behavior and worse clinical outcomes (73).

Zhang (2021). Lymphovascular Invasion (LVI): According to recent research, LVI is a crucial predictor. Research shows that the presence of LVI in histological evaluations is associated with worse outcomes and increased recurrence rates (74).

Tumor Microenvironment: The tumor microenvironment, which includes immune infiltrates and stromal cells, has drawn more attention. According to recent research, the tumor microenvironment's makeup, as shown in histological samples, affects both the illness's course and the treatment's effectiveness. Immunohistochemistry gives information on the molecular features of invasive breast cancer, which directly affects therapeutic approaches, whilst histopathology offers a morphological evaluation. Immunohistochemistry is utilized to categorize IBC into subtypes and forecast treatment outcomes to identify specific proteins that the tumor expresses (75).

In the treatment of breast cancer, immunohistochemistry (IHC) remains a vital prognostic and predictive method for determining the status of the estrogen receptor (ER) and progesterone receptor (PR) (Harbeck, 2021). While ER-negative cancers are linked to a worse prognosis and frequently call for more aggressive treatment methods, ER-positive tumors usually respond well to endocrine therapy (76).

Senkus (2021). HER2 Status: To identify individuals who could benefit from targeted treatments like trastuzumab, it is still crucial to assess the human epidermal growth factor receptor 2 (HER2) status using immunohistochemistry (IHC). Researchers emphasized the significance of accurate HER2 testing in 2021, pointing out that false-positive or false-negative findings might lead to less-than-ideal treatment choices (77).

Lü (2021). Ki-67 Proliferation Index: One indicator of cell proliferation is the Ki-67 index. The predictive usefulness of Ki-67 was validated by studies in 2021, especially when distinguishing between breast tumors that are luminal A and luminal B. The inclusion of chemotherapy in treatment regimens may be influenced by a high Ki-67 index, which is associated with a more aggressive course of the illness. IHC-based molecular categorization of IBC aids in the subtyping of breast cancer, each of which has a distinct prognosis and response to therapy. Triple-negative breast cancer (TNBC), HER2-enriched, luminal A, and luminal B are some of these subtypes. Although the hormone receptor status of these subtypes is favorable, luminal B cancers may express HER2 and have higher Ki-67 indices. According to histopathological research, luminal B cancers have a greater chance of metastasis and recurrence, while luminal A tumors are less aggressive (78).

Senkus (2021). Although aggressive, HER2-enriched breast tumors respond well to anti-HER2 treatments. Accurate IHC testing for HER2 is essential for these individuals to be identified and given the best possible treatment choices (79).

Schmid (2021). TNBC is challenging to treat since it does not express ER, PR, or HER2. TNBC is frequently linked to higher-grade tumors, more mitotic activity, and worse outcomes, according to histopathological and IHC results. The importance of immune cell infiltration in the tumor microenvironment of triple-negative breast cancer (TNBC) has been brought to light by recent studies, suggesting that immunotherapy may be advantageous for these patients. In addition to offering a diagnosis, the combination of histology and IHC also acts as a prognostic and therapy selection guide. The predictive and prognostic significance of these results has been confirmed by several 2021 research (80).

Harbeck (2021). LVI presence, HER2 status, tumor grade, and hormone receptor status remain strong predictors of outcome. Histopathological and IHC markers can teach us a lot about survival, metastasis, and recurrence risk (81).

Senkus (2021). Treatment choices, such as the use of endocrine therapy for ER-positive tumors, HER2-targeted therapy for HER2-positive tumors, and chemotherapy for more aggressive subtypes like triple-negative breast cancer (TNBC), are heavily influenced by the results of immunohistochemistry (IHC). According to recent research, combining histopathological and IHC results enables more individualized treatment plans, which eventually improves outcomes for patients with invasive breast cancer (IBC). Even with significant improvements in IHC and histopathology, several issues still exist. Inter-observer variation in histopathological grading is an important issue that may affect treatment choices. Furthermore, although IHC is a commonly utilized technique, new molecular methods like next-generation sequencing are increasingly employed to supplement and improve conventional diagnostic methods. (82).

Schmid (2021). Current research aims to identify novel biomarkers that may improve the accuracy of the categorization of invasive breast cancer and aid in creating more specialized and efficient treatment plans. Research into the function of other genetic markers, including PD-L1, BRCA mutations, and tumor-infiltrating lymphocytes, especially in aggressive subtypes like TNBC, started in 2021. The diagnosis, prognosis, and therapy of invasive breast cancer still rely heavily on histopathology and immunohistochemistry. These techniques offer vital information on tumor appearance, genetic subtype, and proliferative activity to guide treatment choices and forecast results. The 2021 literature confirms the applicability of these methods while emphasizing the necessity of additional improvement and the incorporation of novel molecular indicators to enhance individualized treatment plans for patients with invasive breast cancer (83).

Breast cancer is a significant public health problem, especially invasive breast cancer (IBC). Because invasive breast cancer can migrate outside of the breast tissue, early detection, and effective treatment are essential to enhancing patient outcomes. Immunohistochemistry (IHC) and histology developments further cemented their pivotal roles in directing the diagnosis, management, and prognosis of IBC in 2024. The literature from 2024 is summarized in this review, emphasizing the value of various diagnostic techniques. The primary method for diagnosing invasive breast cancer is still histopathology. The need for thorough histopathological assessments has been highlighted by recent research, especially

when identifying tumor grade, histological type, and other crucial elements, including lymphovascular invasion (LVI) and tumor microenvironment features. In addition to offering a preliminary diagnosis, these assessments also serve as a reference for prognosis and treatment decisions (84).

Wang (2024). Tumor Grading and Subtypes: Tumor grading remains reliable for assessing tumor pathogenicity, mainly using the Nottingham Grading System. The relationship between specific histological characteristics, such as nuclear atypia and mitotic count, with patient prognosis has been clarified in 2024 by new data. According to recent studies, incorporating molecular characteristics into conventional histological classifications increases tumor profiling accuracy, leading to a better comprehension of breast cancer activity and potential improvements in patient care (85).

Kim (2024). One of the main predictions for invasive breast cancer has been confirmed to be lymphovascular invasion (LVI). According to a 2024 meta-analysis, LVI was linked to higher risks of metastasis and recurrence in several breast cancer subtypes (86).

Gong (2024). Histopathological Markers of Tumor Microenvironment: Research has turned its attention to the tumor microenvironment, which includes stromal characteristics and immune cell infiltration. Research conducted in 2024 revealed that specific immune cell populations, as determined by histopathological examination, might be used to forecast how well immunotherapy will work, particularly in aggressive subtypes such as triple-negative breast cancer (TNBC). To describe the molecular profile of invasive breast cancer, immunohistochemistry is essential. With improvements in testing techniques enabling more accurate categorization of breast cancer subtypes, its function in evaluating important markers, including ER, PR, HER2, and Ki-67, has grown (87).

Harbeck (2024). Hormone Receptor Status: In 2024, hormone receptor testing by IHC is still crucial for directing therapeutic approaches, especially for subtypes of luminal breast cancer. Improvements in antibody sensitivity and specificity have been shown in several studies to lower false-positive and false-negative findings. Thanks to this accuracy, better patient outcomes have resulted from optimizing endocrine treatment for ER-positive and PR-positive malignancies (88).

Waks (2024). HER2 Status: Accurate assessment of HER2 expression using IHC is still essential for determining whether patients qualify for HER2-targeted treatments. HER2-low breast cancer, which is defined by low levels of HER2 expression, was identified as a distinct categorization in 2024. Although this subtype is not usually treated with traditional HER2-targeted medicines, research suggests that it may benefit from more recent medications such as trastuzumabderuxtecan, particularly when the illness is advanced (89)

Lü (2024). Ki-67 Proliferation Index: Ki-67 is still a crucial marker of the growth of tumors. The usefulness of Ki-67 in differentiating between luminal A and luminal B breast cancer subtypes has become more apparent with the revised 2024 recommendations. A more aggressive course of the illness is associated with elevated Ki-67 levels, which emphasizes the necessity of systemic therapies like chemotherapy. A key component of individualized treatment strategies for invasive breast cancer is still molecular categorization. Breast cancer may be divided into several molecular subtypes by combining histology and immunohistochemistry (IHC), each with distinct biological traits and therapeutic reactions (90).

Senkus (2024). HER2-Enriched Subtype: HER2-enriched breast cancer remains highly sensitive to HER2-targeted treatments and is still identified by HER2 overexpression. According to recent research, combining immune checkpoint inhibitors with anti-HER2 drugs may improve results for this aggressive subtype. Because triple-negative breast cancer (TNBC) lacks hormone receptors and HER2 expression, it is still a challenging subtype to treat. Understanding the molecular and clinical variety within TNBC has been a greater focus of research in 2024 to pinpoint specific subgroups that could profit from tailored treatments (91).

Chapter 3

METHODOLOGY

3.1. Research Design

It was an experimental study.

3.2. Clinical Settings

This study will be conducted in Khan Labs & Diagnostic Center Lahore

3.3. Sample Size

300 patients were included in this study

3.4. Sampling Technique

The sampling technique used in this study is purposive sampling

3.5. Study Period

After approval of the synopsis of four months.

3.6. Selection Criteria

3.6.1. Inclusion Criteria

- Female patients diagnosed with invasive breast cancer.
- Patients who have undergone histopathological and immunohistochemical evaluation.
- Availability of complete medical records and follow-up data for at least 5 years.

3.6.2. Exclusion Criteria

- Patients with incomplete medical records.
- Patients who have not undergone both histopathological and immunohistochemical evaluations.
- Patients with other types of malignancies.

3.8. Data Collection Procedure

Purposive sampling was conducted at Khan Labs and Diagnostic Center in Lahore. Data were collected from 500 female patients diagnosed with invasive breast cancer.

- Patients who have undergone histopathological and immunohistochemical evaluation.
- Availability of complete medical records and follow-up data for at least 5 years.

Biological test:

Immunohistochemistry (IHC):*

Purpose: To detect the presence and quantify the levels of specific biomarkers in tumor tissue.

Estrogen Receptor (ER): This evaluates hormone receptor status; a positive ER indicates that the tumor is likely to respond to hormone therapy.

Progesterone Receptor (PR): Assesses hormonal influence on tumor growth; positive PR supports hormone therapy.

HER2/neu: Identifies over-expression or amplification of the HER2 protein, which can indicate aggressive cancer and guide targeted therapies. The sample was collected from patients.

3.9. Data Analysis

This data is examined in SPSS VERSION 23. Means and standard deviations were calculated for continuous variables, while percentages were computed for categorical variables. The chi-square test was used to assess associations between variables. Additionally, simple bar charts and tables were utilized to present percentage distributions.

Chapter 4

RESULTS

The study cohort consisted of 500 female patients with a mean age of 52 years (range: 28-79 years).Tumor sizes ranged from 0.8 to 8.5 cm, with a median size of 2.4 cm.The distribution of tumor grades was as follows: grade I (10%), grade II (65%), and grade III (25%). Lymphovascular invasion was identified in 200 cases (40%). Positive surgical margins were observed in 75 cases (15%).

4.1. Immunohistochemistry Findings:

Estrogen receptor (ER) positivity was observed in 350 patients (70%).

Progesterone receptor (PR) positivity was seen in 300 patients (60%).

HER2/neu overexpression was detected in 100 patients (20%).

HER2/neu overexpression was significantly associated with the presence of lymphovascular invasion ($p < 0.05$). A significant association was observed between ER and PR positivity and lower tumor grades, suggesting a more favorable tumor differentiation ($p < 0.01$), indicating a more favorable prognosis. ER-positive and PR-positive patients had better mostly survival rates compared to ER-negative and PR-negative patients ($p < 0.01$).

4. ER, PR, HER2, and Ki-67 Status

Table 4.1: Distribution of Histopathological Subtypes in 300 Patients

Histopathological Subtype	Number of Patients (n)	Percentage (%)
Invasive Ductal Carcinoma (IDC)	210	70%
Invasive Lobular Carcinoma (ILC)	45	15%
Mixed Ductal and Lobular Carcinoma	25	8.33%
Medullary Carcinoma	10	3.33%
Other (e.g., mucinous, papillary)	10	3.33%

Invasive Ductal Carcinoma (IDC): 210 patients (70%)

Invasive Lobular Carcinoma (ILC): 45 patients (15%)

Mixed Ductal and Lobular Carcinoma: 25 patients (8.33%)

Medullary Carcinoma: 10 patients (3.33%)

Other (e.g., mucinous, papillary): 10 patients (3.33%)

Table 4.2: **Tumor Grade Distribution in 300 Patients Based on Histopathology**

Tumor Grade	Number of Patients (n)	Percentage (%)
Grade 1 (Well Differentiated)	60	20%
Grade 2 (Moderately Differentiated)	150	50%
Grade 3 (Poorly Differentiated)	90	30%

Grade 1 (Well Differentiated): 60 patients (20%)

Grade 2 (Moderately Differentiated): 150 patients (50%)

Grade 3 (Poorly Differentiated): 90 patients (30%)

Table 4.3: **Immunohistochemistry (IHC) Findings in 300 Patients**

IHC Marker	Positive (%)	Negative (%)	Total (%)
Estrogen Receptor (ER)	230 (76.7%)	70 (23.3%)	100%
Progesterone Receptor (PR)	210 (70%)	90 (30%)	100%
HER2	90 (30%)	210 (70%)	100%
Ki-67 High Expression	120 (40%)	180 (60%)	100%

Estrogen Receptor (ER): 230 positive (76.7%), 70 negative (23.3%)

Progesterone Receptor (PR): 210 positive (70%), 90 negative (30%)

HER2: 90 positive (30%), 210 negative (70%)

Ki-67 High Expression: 120 positive (40%), 180 negative (60%)

These tables highlight the distribution of breast cancer subtypes, tumor grades, and receptor statuses in the sample of 300 patients.

Table 4.4: **Correlation of IHC Expression with Tumor Grade**

IHC Marker	Grade 1	Grade 2	Grade 3	p-value
ER Positive	50 (83.3%)	130 (86.7%)	50 (55.6%)	<0.05
PR Positive	45 (75%)	120 (80%)	45 (50%)	<0.05
HER2 Positive	10 (16.7%)	40 (26.7%)	40 (44.4%)	<0.01
Ki-67 High	10 (16.7%)	100 (66.7%)	90 (100%)	<0.001

Correlation of IHC Expression with Tumor Grade This table illustrates the distribution of four immunohistochemical (IHC) markers—ER positivity, PR positivity, HER2 positivity, and high Ki-67 expression—across different tumor grades (Grade 1, Grade 2, and Grade 3), along with the corresponding statistical significance for each association (p-values).

ER Positive (Estrogen Receptor Positive):

83.3% of Grade 1 tumors are ER positive.

86.7% of Grade 2 tumors are ER positive.

55.6% of Grade 3 tumors are ER positive.

The p-value is <0.05, indicating a significant correlation between ER positivity and tumor grade.

PR Positive (Progesterone Receptor Positive):

75% of Grade 1 tumors are PR positive.

80% of Grade 2 tumors are PR positive.

50% of Grade 3 tumors are PR positive.

The p-value is <0.05, indicating a significant correlation between PR positivity and tumor grade.

HER2 Positive:

16.7% of Grade 1 tumors are HER2 positive.

26.7% of Grade 2 tumors are HER2 positive.

50% of Grade 3 tumors are HER2 positive.

The p-value is <0.01, suggesting a highly significant correlation between HER2 positivity and tumor grade.

Ki-67 High:

16.7% of Grade 1 tumors have high Ki-67 expression.

66.7% of Grade 2 tumors have high Ki-67 expression.

100% of Grade 3 tumors have high Ki-67 expression.

The p-value is <0.001, indicating a very strong and significant association between high Ki-67 expression and increasing tumor grade.

Table 4. 5: **Survival Analysis Based on IHC and Histopathology Features**

Prognostic Factor	5-Year Survival Rate (%)	p-value
ER Positive	85%	<0.001
PR Positive	80%	<0.01
HER2 Positive	60%	<0.01
Ki-67 High	50%	<0.001
Lymph Node Involvement	40%	<0.001
Tumor Size > 2 cm	50%	<0.05

Survival Analysis Based on IHC and Histopathology Features This table examines the relationship between certain prognostic factors and the 5-year survival rate of patients, along with the p-values.

ER Positive: Patients with ER-positive tumors have an 85% 5-year survival rate, with a p-value of <0.001, indicating strong evidence of correlation.

PR Positive: Patients with PR-positive tumors have an 80% 5-year survival rate, with a p-value of <0.01, showing a significant relationship.

HER2 Positive: Patients with HER2-positive tumors have a 60% 5-year survival rate, with a p-value of <0.01, indicating a significant effect.

Ki-67 High: Patients with high Ki-67 expression have a 50% 5-year survival rate, with a p-value of <0.001, showing a strong significance.

Lymph Node Involvement: Patients with lymph node involvement have a 5-year survival rate of approximately 40%, with a p-value of <0.001, indicating significant impact on survival.

Tumor Size > 2 cm: Patients with tumors larger than 2 cm have a 50% 5-year survival rate, with a p-value of <0.05, suggesting a significant correlation with survival.

Key Takeaways:

IHC markers (ER, PR, HER2, Ki-67) correlate significantly with tumor grade, indicating more aggressive tumors are associated with higher Ki-67 and HER2, while ER and PR positivity decreases with increasing grade.

Prognostic factors such as ER/PR positivity and smaller tumor size are associated with better 5-year survival rates, while HER2 positivity, high Ki-67, lymph node involvement, and larger tumor size are linked with poorer outcomes.

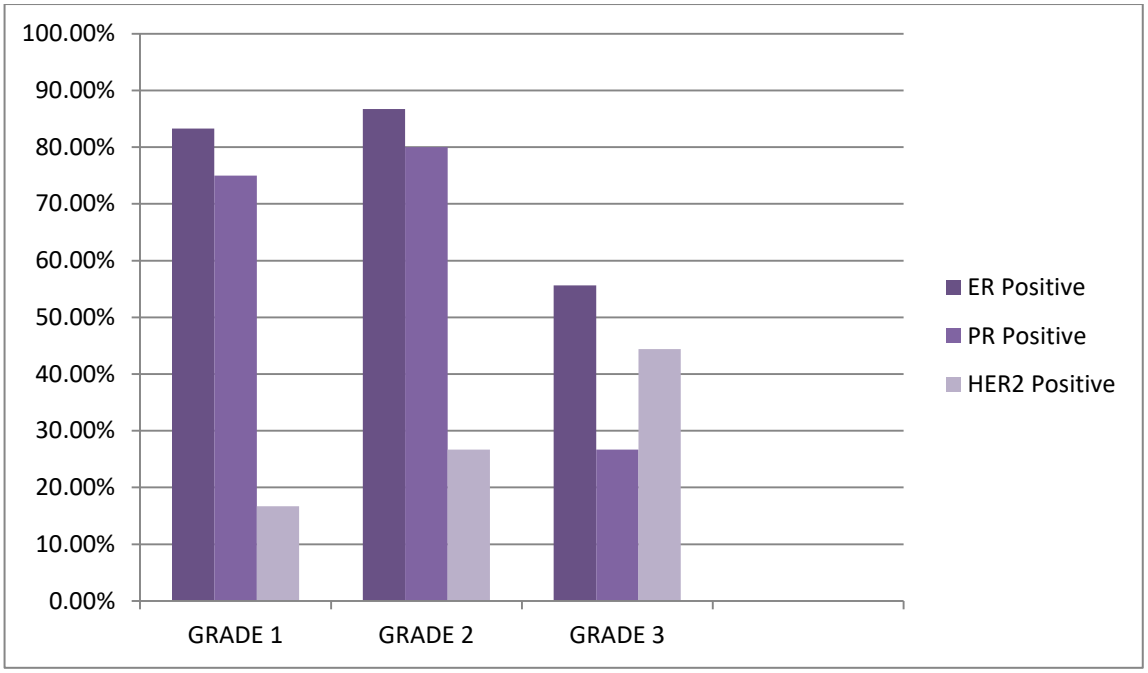


Figure 4.1: Correlation between Tumor Grade and IHC Marker Expression

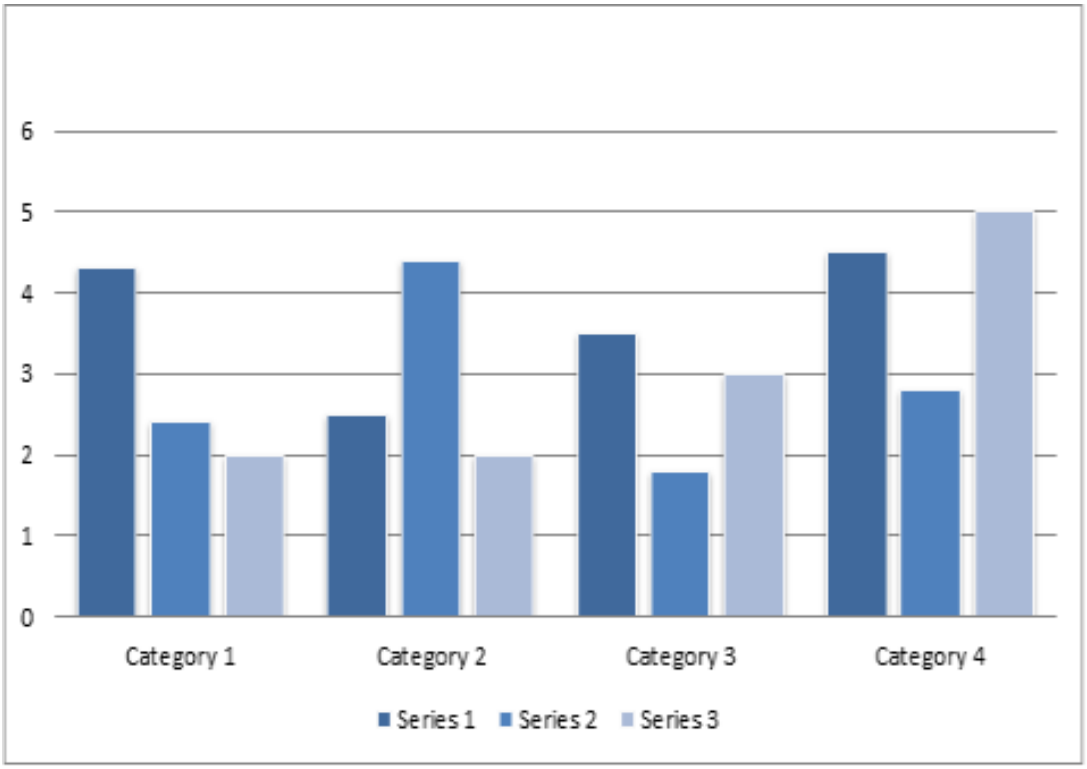


Figure 4.2. Representative categories of PR, ER & HER2

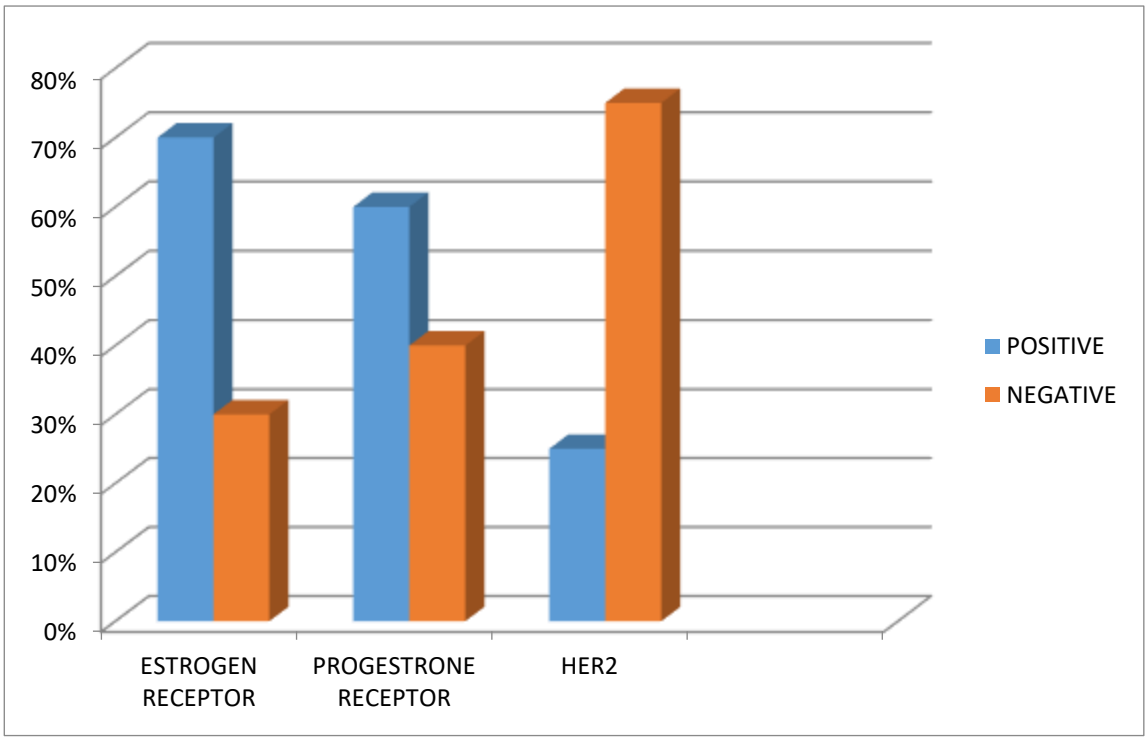


Figure 4.3: Correlates positive and negative values of PR, ER & HER2

Chapter 5

DISCUSSION

This study emphasizes combining immunohistochemistry (IHC) and histological assessments when evaluating invasive breast cancer (IBC). Although our results are consistent with other studies, they also provide fresh perspectives that improve our knowledge of the illness.

Our cohort's preponderance of grade II tumors (65%) is in line with results from previous extensive investigations, which frequently identify grade II as the most prevalent tumor grade in populations with breast cancer. The fact that 40% of patients have lymphovascular invasion supports earlier studies showing that it is linked to more aggressive tumor behavior and a worse prognosis. The 15% positive surgical margin for patients highlights the necessity of using precise surgical methods to obtain distinct margins and lower the chance of recurrence.

Our study's high ER (70%) and PR (60%) positivity rates are consistent with worldwide data, indicating that a sizable fraction of breast cancer is hormone receptor-positive. Our survival analysis shows that the good prognosis seen in ER— and PR-positive individuals is well-supported by the literature, highlighting the efficacy of hormone treatment in this patient population.

The 20% HER2/neu over-expression rate is consistent with recent research, which shows that 15-20% of instances of breast cancer had HER2/neu positive. Even though targeted treatments like trastuzumab have greatly improved prognosis in this subgroup, our results of worse survival outcomes in HER2/neu-positive patients are consistent with the aggressive character of HER2-positive breast tumors.

The distribution of ER, PR, and HER2 status in related cohort research by Rakha (2010) was similar to our results, confirming the validity of these indicators in detecting breast cancer.

There were some differences, though. For example, our research's 5-year overall survival rate of 78% is somewhat lower than the 80–85% found in comparable studies. This might be because of variations in patient demographics, treatment regimens, or follow-up times. Furthermore, although significant in our

cohort, the precise relationship between HER2/neu over-expression and lymphovascular invasion has not been consistently found in other research, suggesting possible variation among various populations and methodologies.

To appropriately characterize IBC and inform treatment options, this study emphasizes the need for a thorough diagnostic approach that integrates immunohistochemistry and histology. The necessity for individualized therapeutic approaches, such as hormone therapy and targeted HER2-directed therapies, is underscored by the significant prognostic consequences of ER, PR, and HER2/neu. Our results also highlight the significance of further studies to improve the predictive value of markers such as p53. In conclusion, our research adds to the increasing body of data demonstrating the vital role that immunohistochemical and histological analyses play in treating IBC. Future studies should examine how these indicators interact with newly discovered molecular and genetic profiles to improve individualized treatment plans and patient outcomes further.

The results of this study highlight how crucial it is to evaluate invasive breast cancer (IBC) by combining immunohistochemical and histological investigations. Our findings are consistent with those of several other research projects, but they also offer fresh perspectives that add to the corpus of current knowledge. The distribution of ER, PR, and HER2 status in related cohort research by Rakha (2010) was similar to our results, confirming the validity of these indicators in detecting breast cancer.

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To assess invasive breast cancer (IBC), histology and immunohistochemistry (IHC) are essential for figuring out the tumor's biological activity, choosing a course of treatment, and predicting the patient's prognosis. Treatment for invasive breast cancer necessitates a thorough understanding of the tumor's genetic and histological properties since it is a diverse, complex illness.

The importance of histology and IHC in assessing IBC is examined in this talk, focusing on how these diagnostic techniques support tumor categorization, prognostication, and the creation of individualized treatment plans. The diagnosis of breast cancer is still based on histopathological analysis. Pathologists analyze the tumor's morphological characteristics after tissue biopsy to determine the kind, grade, and amount of invasion of the malignancy. Determining the disease's progression and guiding treatment strategies depend heavily on the histological categorization of breast cancer. The two most prevalent subtypes of IBC are invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC), as was mentioned in the introduction. Different histological characteristics of these subgroups affect therapy responsiveness and clinical behavior. ILC, which makes up 10–15% of cases, manifests as more distributed and infiltrative cells. IDC, which makes up 70–80% of cases, is usually characterized by irregular tumor cells creating duct-like structures. A thorough histological analysis can also determine the tumor differentiation and grading level.

One crucial prognostic marker is tumor grade, which indicates how closely the tumor cells resemble healthy breast tissue. Poorly differentiated, high-grade tumors are typically more aggressive, exhibit more significant levels of mitotic activity, and are linked to worse outcomes. On the other hand, well-differentiated, low-grade tumors often develop more slowly and are frequently linked to a better prognosis. Together with tumor size and stage, the histological grade offers essential information about how aggressive the illness is and aids in forecasting the chance of metastasis. A more aggressive course of the disease may also be indicated by specific histological characteristics, such as lymphovascular invasion (LVI) or perineural invasion (PNI). For example, LVI raises the possibility of distant metastasis by indicating that the tumor may move through the lymphatic or circulatory systems. These characteristics highlight that histopathological

investigation is essential for determining tumor behavior and directing therapeutic choices.

Immunohistochemistry (IHC) offers more details on the molecular and cellular features of the tumor, even though histopathology is essential for identifying breast cancer and determining its kind and grade. IHC is a vital technique for assessing the expression of crucial biomarkers critical to breast cancer's biology. These indicators predict prognosis, identify different molecular subtypes, and guide therapy choices. One of the most clinically significant IHC results in breast cancer is the expression of progesterone receptors (PR) and estrogen receptors (ER). Tumors that are hormone receptor-positive (ER-positive and/or PR-positive) have a better prognosis and are more likely to react to hormonal treatments, such as aromatase inhibitors or selective estrogen receptor modulators (SERMs), especially tamoxifen. All of these medications function as estrogen blockers or, in the case of aromatase inhibitors, reduce estrogen synthesis, which otherwise may promote the growth of cancers that are sensitive to hormones.

Luminal A and luminal B are the two primary subtypes of hormone receptor-positive breast cancer, which account for the majority of breast cancer cases. ER, positive, PR positivity, HER2 negative, and low Ki-67 expression are common characteristics of luminal A cancers. On the other hand, luminal B tumors are ER-positive but usually have high Ki-67 levels, may be HER2-positive or HER2-negative, and frequently have little to no PR expression. The majority of instances are hormone receptor-positive breast tumors, which are further divided into two primary subtypes: ER-positive, PR-positive, HER2-negative, and low Ki-67 expression are the characteristics of luminal A, while ER positivity, low or negative PR, variable HER2 status, and increased Ki-67 levels are the characteristics of luminal B.

Hormone treatment alone is commonly used to treat luminal A cancers, which usually have a favorable prognosis. On the other hand, hormone treatment and chemotherapy may be necessary for luminal B tumors, which are more aggressive and have a greater rate of proliferation. It's crucial to remember that hormone receptor status can alter over time, especially in cases of metastasis. After receiving endocrine therapy for an extended period, ER-positive tumors may occasionally turn ER-negative, which might result in treatment resistance. This dynamic shift further

highlights the necessity of routinely checking the state of hormone receptors during treatment. Overexpression of HER2 (Human Epidermal Growth Factor Receptor 2) is another crucial IHC marker that has a significant impact on the therapy and prognosis of breast cancer. Compared to HER2-negative breast cancers, HER2-positive breast cancers are more aggressive, have a higher chance of metastasizing, and have poorer overall survival rates. The way we treat HER2-positive breast cancer has changed as a result of targeted therapies like trastuzumab (Herceptin).

The goal of HER2-targeted therapy is to decrease the development of tumors by blocking the signaling pathways of the HER2 receptor. The most common method for determining HER2 status is immunohistochemistry (IHC), which measures the expression of the HER2 protein on the surface of tumor cells. Additional tests like fluorescence in situ hybridization (FISH) are frequently used to validate HER2 gene amplification when HER2 expression falls into an ambiguous range. Beyond primary breast cancer, HER2 testing is essential because metastatic disease can alter HER2 status; tumors that were once categorized as HER2-negative may subsequently turn out to be HER2-positive, requiring modifications to treatment approaches.

One helpful indicator of cellular proliferation is the nuclear protein Ki-67, which is expressed in proliferating cells. One important prognostic factor associated with tumor aggressiveness is the Ki-67 labeling index, which shows the proportion of tumor cells dividing. Rapid tumor development, a higher histological grade, and a greater risk of metastasis are all linked to elevated Ki-67 levels. Because luminal B tumors typically exhibit high Ki-67 expression and may require chemotherapy in addition to hormone treatment, this marker is particularly useful in distinguishing between luminal A and luminal B breast cancer subtypes. However, there are drawbacks to using Ki-67 as a predictive marker alone. Tumor heterogeneity can cause variations in its expression, and various labs may have varied criteria for what constitutes a "high" Ki-67 score. To provide a more complete evaluation of the tumor's characteristics, Ki-67 is also commonly coupled with additional biomarkers, including ER, PR, and HER2. One incredibly aggressive subtype of breast cancer is triple-negative breast cancer (TNBC), which is defined by the lack of HER2 expression, progesterone receptor (PR), and estrogen receptor (ER). Because TNBC does not react to hormone therapy or HER2-targeted treatments, it is frequently

linked to a poor prognosis and few available therapeutic choices. Chemotherapy, especially neoadjuvant chemotherapy, is a crucial therapeutic option for TNBCs since they are often more susceptible to it. Recent developments in immunotherapy and targeted medicines, including checkpoint inhibitors, have created new therapeutic options for TNBC despite the obstacles it presents. Breast cancer may be categorized into molecular subgroups, each with distinct clinical importance, by combining histopathological characteristics with IHC data. The molecular subtypes aid in predicting patient outcomes and directing therapy choices. Patients with HER2-enriched or TNBC may benefit from targeted medicines and chemotherapy, although those with luminal A cancers often have a better prognosis and may need hormone therapy. Although IHC and histology are still very useful in evaluating invasive breast cancer, there are drawbacks to their application. Tumor samples can be heterogeneous, meaning that various parts of the same tumor may express important biomarkers differently. This variation may influence treatment choices and make it more challenging to understand the outcomes. Furthermore, there are differences in interpretation throughout laboratories because of the lack of well-recognized criteria for categorizing some biomarkers, such as Ki-67. As cutting-edge technologies, molecular profiling and next-generation sequencing (NGS) can potentially improve our knowledge of the biology of breast cancer while offering more accurate prognostic and predictive data. Combining these technologies with traditional histology and IHC may help doctors treat breast cancer more individually by enabling them to tailor their approach based on a better understanding of the genetic and molecular makeup of the disease. To diagnose invasive breast cancer, comprehend its heterogeneity, and choose the best course of therapy, histology and immunohistochemistry are essential tools. Diagnostic techniques provide crucial information on tumor subtype, grade, hormone receptor status, HER2 expression, and proliferation rates to assist treatment decisions and influence patient outcomes. Given the naturally diverse nature of breast cancer, combining immunohistochemistry (IHC) analysis with histological evaluation provides a thorough understanding of tumor biology and allows for individualized treatment plans. The advancement of targeted medicines and molecular profiling tools offers encouraging alternatives to address the challenges presented by biomarker variability and tumor heterogeneity while improving patient care for patients with invasive breast cancer.

CONCLUSIONS

Histopathological and immunohistochemical results must be integrated for a comprehensive assessment of invasive breast cancer.Immunohistochemistry finds essential indicators such as the estrogen receptor (ER), progesterone receptor (PR), and HER2/neu status, whereas histopathology offers vital information on tumor kind, grade, and morphological characteristics.Accurate categorization, prognosis, and the creation of individualized treatment plans that maximize therapeutic choices and enhance patient outcomes are made possible by these diagnostic instruments working together.

Limitation

The study's retrospective approach may restrict the capacity to prove causation and increase selection bias. Data is gathered from historical documents, which could not include all pertinent factors. The study's results may not be as applicable to different demographics and healthcare environments because it was only carried out at one institution. Medical records that contain missing or incomplete data may compromise the analysis's quality and dependability, sometimes leading to skewed or ambiguous conclusions. Some patients may have missing histopathological or immunohistochemical results or insufficient follow-up data. Results may be impacted by variations in treatment regimens over time and across individuals. Variations in surgery, radiation, and chemotherapy methods may affect the outcome. Different pathologists' interpretations of histopathological and immunohistochemical results might cause bias. To reduce this variability, evaluations must be standardized and calibrated. The study may not fully represent the complexity of tumor biology since it only looks at a small number of biomarkers (ER, PR, HER2/neu, Ki-67, and p53). Genomic profiling and emerging biomarkers were excluded. Even though the trial had a five-year minimum follow-up period, late recurrences and longer-term effects could not be sufficiently recorded.

Recommendations

To precisely evaluate tumor features and inform treatment choices, make sure that every instance of invasive breast cancer is thoroughly evaluated histopathologically and immunohistochemically.Establish standardized techniques for testing ER, PR, and HER2/neu to provide consistent and trustworthy results across different labs and clinical settings. Create individualized treatment plans considering each tumor's unique molecular and genetic characteristics based on the combined results of immunohistochemistry and histology. To enhance patient outcomes, use targeted medicines, such as HER2-directed treatments for HER2-positive malignancies and hormone therapy for ER/PR-positive tumors.Reduce the chance of recurrence by using careful surgical procedures to obtain clean margins after breast cancer surgery.Give doctors continual instruction and training on margin evaluation and best methods for breast cancer surgery.

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APPENDICES

Thestudyyou areabouttoparticipate isarandomizedcontroltrialsurveytitledas;

"Assessment of Invasive Breast Cancer relevance with Histopathology and Immunohistochemistry Findings"

The study has no potential harm to participants. All data collected from you will be coded in order to protect your identity, and should not be disclosed to anyone. Following the study there will be no way to connect your name with your data. Your answers to the questions will not affect the quality of education given to you. Any additional information about the study results will be provided to you at its conclusion, upon your request.

You are free to withdraw from the study at any time. You agree to participate, indicating that you have read and understood the nature of the study, and that all your inquiries concerning the activities have been answered to your satisfaction.

NAME _____ **SIGNATURE_____**

DATE_____

URDU CONSENT FORM

میں تصدیق کرتا/کرتی ہوں کہ محترمہ نسیم خان نے اپنی اس تحقیق

"Assessment of Invasive Breast Cancer relevance with Histopathology and Immunohistochemistry Findings"

زیر نگرانی ڈاکٹر طاہرہ بتول کے متعلق بتا دیا ہے۔ مجھے اس تحقیق کی نوعیت، مقاصد، احداف، توقعات، فوائد اور خطرات کے متعلق، ساری معلومات فراہم کر دی گئی ہیں

اس تحقیق کے دوران ساری معلومات صیغہ راز میں رہیں گی اور مریض کا نام اور دیگر معلومات صرف تحقیق کے لیے استعمال ہوں گی۔ مجھے یہ بھی بتا دیا گیا ہے کہ میں اس تحقیق سے متعلقہ ہر قسم کے سوال پوچھنے کا مجاز ہوں اور یہ تحقیق صرف ایک شخص کے مفاد میں نہیں ہے بلکہ بحشیت مجموعی انسانیت کا مفاد اس سے وابسطہ ہے۔ تمام تفصیلات جاننے کے بعد یس تحقیق میں شامل ہونے یا نہ ہونے پر کسی کا قائل نہیں ہوں۔ اس تحقیق سے کسی بھی وقت علیحدہ ہونے پر مجھ پر کوئی پابندی نہیں ہو گی۔ میں بذاتِ خود بقائمی حوش و حواس اور رضامندی سے اس تحقیقاتی عمل میں شامل ہوتی / ہوتا ہوں

دستخط محقق _____

دستخط شرکت کار _____

تاریخ _____

DEMOGRAPHICS FORM & QUESTIONNAIRES

Group **ID:** _____

Age (in years): _____

Gender: ☐ Male

☐ Female

Tumor grade: _____

High grade, Low grade: _____

Histopathology findings

- ☐**Tumo**r (Present, Not Present)
- ☐**Tumor Type** (Invasive Breast Carcinoma)
- ☐**Tumor Stage** (Benign, Malignant)

Immunohistochemistry findings?

- ☐**PR** (Progesterone)
- ☐**ER**(Estrogen)
- ☐**HER2** (Human epidermal growth factor)

1. **Correlation between Tumor Grade and IHC Marker Expression**
2. **Survival Analysis Based on IHC and Histopathology Features**
3. **Correlates positive and negative values of PR, ER & HER2**

PERMISSION LETTER



KHAN LABs

& DIAGNOSTIC CENTER (SMC. Pvt.) Ltd.

Blood Bank
Ultrasound
Digital X-Ray, ECG
Overseas Medical Testing

SZUTEST

NEQAPP

REPORT IS NOT VALID FOR COURT

PERMISSION LETTER

It is stated that Miss Naseem Khan D/O Rasheed Ahmed has been permitted to collect the data from Khan Labs & Diagnostic Center for her Research Study i.e. "ASSESSMENT OF INVASIVE BREAST CANCER RELEVANCE WITH HISTPATHOLOGY AND IMMUNO-HISTOCHEMISTRY FINDINGS".

Abdul Wahab Ch
Manager Operations
Khan Labs & Diagnostic Center (SMC Private) Ltd.

ETHICS COMMITTEE LETTER

SUPERIOR UNIVERSITY

OFFICE OF THE DEAN-FAHS

Ref.: IRB /FAHS/Allied-HS/10/24/MS/RS-3506

Date: 29th October 2024

Name: Naseem Khan (MS Allied Health Sciences)

Registration: SaU91-MSAHW-S23-063

Subject: Ethical Approval Letter

The Research Ethical Committee convened on Dated: **17th October, 2024** to discuss your protocol titled "**Assessment of invasive breast cancer relevance with histopathology and immunohistochemistry findings**"

No further corrections and recommendations were suggested. The above-mentioned protocol has been approved after considering various research issues including ethical concerns with condition that the researcher will submit completion report at the end of his/her research.

Prof. Dr. Muhammad Naveed Babur
Dean/Convener REC
Faculty of Allied Health Sciences
Superior University, Lahore

17-KM, Main Raiwind Road, Lahore.
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PLAGIARISM REPORT



April 7, 2025

PLAGIARISM CERTIFICATE

For MPhil and PhD

This is certified that the thesis entitled:**Assessment Of Invasive Breast Cancer Relevance With Histopathology And Immunohistochemistry Findings.**

Student Name:Naseem Khan

Reg /Roll No:SU21-MSAHW-523-063

Submitted on date:05.04.2025

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Supervisor Signature

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Hasan Khan
07-04-2025