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Molecular and Histopathological Advances in Breast Cancer Diagnosis: Integrating AI and Biomarker Profiling for Personalized Therapeutics in 2025

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ABSTRACT

Background: Breast cancer is the most frequently diagnosed cancer in women worldwide. Early and precise diagnosis is important to maximize the outcomes of treatment. The purpose of this research was to combine histopathological assessment, molecular biomarker profiling, and artificial intelligence (AI)-aided digital pathology to increase diagnostic accuracy and therapeutic targeting of breast cancer patients.
Methods: 60 female breast cancer patients admitted in Bahawal Victoria Hospital, Bahawalpur, were included. Histological grading, immunohistochemistry (ER, PR, HER2, Ki-67), and molecular analysis (BRCA1/2, TP53, PIK3CA, EGFR) were done. AI algorithms were used to evaluate digital histopathology slides for tumor classification, mitotic activity, and Ki-67 scoring. Statistical correlations among molecular, pathological, and AI parameters were assessed.
Results: The most prevalent subtype was invasive ductal carcinoma (63.3%). Tumors with high grade had high Ki-67 index, TP53 mutation, and aggressive phenotype. Triple-negative breast cancer was diagnosed in 16.7% of the cases. AI-aided analysis achieved 88% grade accuracy and 93% Ki-67 score agreement compared with conventional assessments. Molecular profiling identified clinically actionable mutations in 40% of patients.
Conclusion: The incorporation of conventional pathology, molecular diagnosis, and AI-based analysis greatly augments breast cancer assessment and management. This inter-disciplinary model facilitates individualized therapeutic intervention, especially in resource-constrained healthcare environments, and is an encouraging step forward in precision oncology.

Introduction

Breast cancer is still the most common cancer among women globally, posing an enormous health system burden. Breast cancer is estimated by the World Health Organization (WHO) to cause about 2.3 million new cases each year, with increasing incidence in both developed and developing countries [1]. In spite of the impressive progress in imaging, detection, surgical procedures, and systemic treatments, breast cancer mortality is still unacceptably high, particularly in low-resource environments where early diagnosis tends to be overlooked. The molecular, genetic, and histological heterogeneity of breast cancer requires a more specific strategy in its diagnosis and treatment [2]. Conventional diagnostic approaches, which are primarily image- and histopathology-based, tend to lack the level of granularity needed to inform personalized therapeutic options. With the coming new era in the fast-changing world of oncology, comingling with AI and molecular biomarker profiling signifies a new era in the diagnostics and personalized therapeutics of breast cancer [3].

By 2025, the diagnostic model is experiencing a significant shift, influenced by the crossroads of digital pathology, omics technologies, and AI-driven data analysis. Bringing these technologies together, a richer picture of the biology of breast cancer is being created with unprecedented accuracy to identify subtypes, forecast disease progression, and customize treatment regimens to the individual molecular footprint of the tumor [4]. Identification and validation of prognostic and predictive biomarkers from DNA mutations and RNA expression signatures to proteomic and metabolomic profiles have set the stage for personalized medicine. In parallel, AI algorithms are transforming histopathological workflows by enhancing human capabilities, lessening inter-observer variability, and improving reproducibility of diagnostic results [5].

Histopathologic evaluation, traditionally the gold standard for diagnosing breast cancer, is expanding beyond its historical limits. With the digitization of whole-slide images (WSIs) and the creation of AI-facilitated image analysis software, pathologists are now better able to determine tissue architecture, mitosis, nuclear atypia, and architectural pattern with increased accuracy and efficiency [6]. Deep learning models, specifically convolutional neural networks (CNNs), have demonstrated outstanding performance in identifying invasive and in situ lesions, tumor grading, and even molecular subtype prediction based on histological appearance. These breakthroughs are not just complementing conventional pathology but are transforming it into an ever more quantitative and reproducible discipline [7].

Concurrently, molecular diagnostics has proven to be a key field in the stratification of breast cancer. Genomic tests like Oncotype DX, MammaPrint, and PAM50 have been a game-changer in assessing the risk of recurrence and making decisions about adjuvant therapy. Refinements in next-generation sequencing (NGS) technologies have allowed exhaustive mutation profiling in core oncogenes and tumor suppressor genes like BRCA1, BRCA2, TP53, PIK3CA, and HER2 [8]. Epigenetic modifications, microRNA expression, and tumor microenvironment markers are being studied for their implications in the pathogenesis of breast cancer as well as resistance to therapy. Combining these molecular data with histopathological features gives a more complete impression of the disease and hence makes the choice of targeted therapies like HER2 inhibitors, CDK4/6 inhibitors, and PARP inhibitors more informative [9].

Artificial intelligence plays the role of the binding glue that combines molecular and histopathological information into a consistent diagnostic framework. By means of machine learning and deep learning algorithms, AI is able to process enormous datasets produced by genomic sequencing, digital pathology, and clinical data to detect patterns and correlations otherwise invisible to human inquiry [10]. These AI models can generate significant predictive models of response to treatment, survival, and possible toxicities and side effects, thus aiding oncologists in making informed decisions. Expert platforms powered by AI can also learn from new data continuously, enhance their predictive power with time, and adjust to new biomarkers or treatment modalities[11].

Perhaps the most promising area for AI usage in breast cancer diagnosis lies in radiogenomics, where imaging characteristics are compared with genomic profiles [12]. For example, AI can use mammographic or MRI characteristics to predict the occurrence of certain mutations or receptor statuses (ER, PR,

HER2), thus decreasing the dependency on invasive biopsies. Similarly, AI-enhanced digital pathology is able to forecast molecular subtypes like luminal A, luminal B, HER2-enriched, or triple-negative with high accuracy based on morphology alone. These minimally invasive or non-invasive diagnostic technologies not only enhance patient comfort but also enable more rapid and more frequent monitoring of disease or therapeutic response [13].

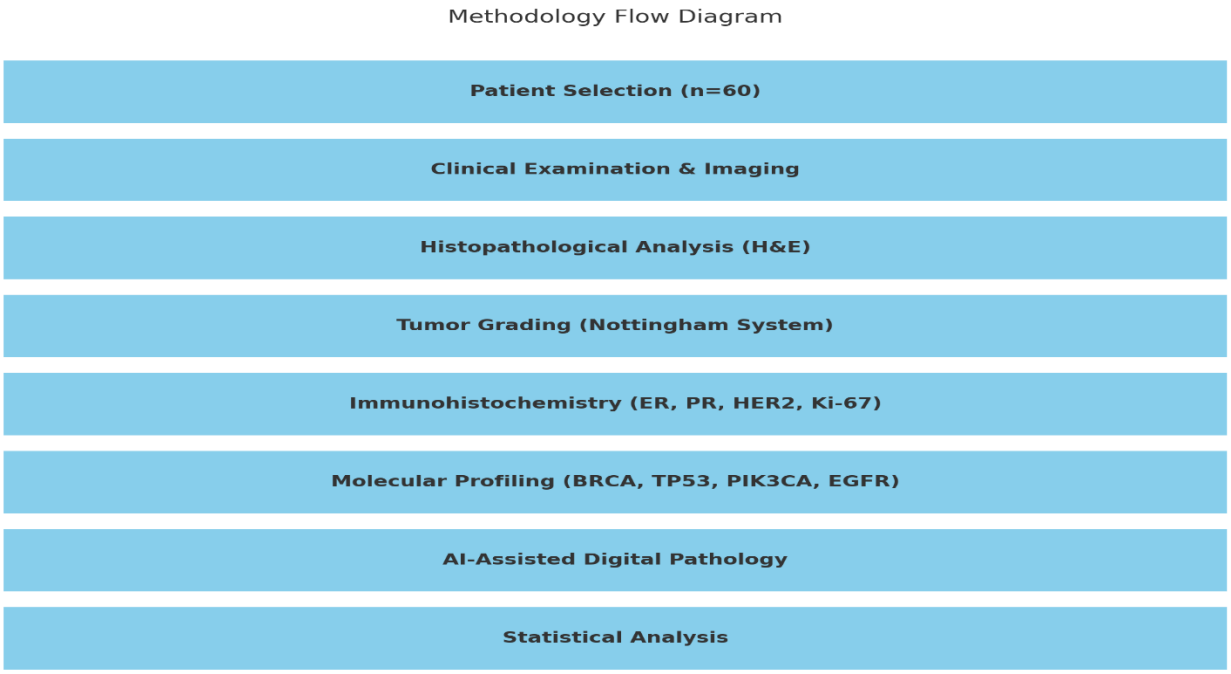
Even with these advances, effective application of AI and molecular profiling into everyday clinical practice is riddled with obstacles. The integration of data is a major impediment, as information from histopathology, genomics, radiology, and clinical history tends to be in siloed systems [14]. Data format standardization, platform interoperability, and ethical issues of data privacy and algorithmic explainability need to be addressed. Further, implementation of AI tools necessitates redesigning medical training and education to enable clinicians and pathologists to interpret and validate AI output. Regulatory guidelines also need to be developed to guarantee the safety, effectiveness, and responsibility of AI-based diagnostic machines [13].

Within the frame of personalized medicine, AI-driven molecular and histopathological diagnostics are facilitating precision oncology through the incorporation of molecular diagnostics. Instead of being treated on a one-size-fits-all basis, patients are now treated based on specific profiles accounting for genetic mutations, tumor heterogeneity, metabolism, and immune state. Clinical trials are also conforming to this paradigm with basket trials, umbrella trials, and adaptive designs enrolling patients by molecular features rather than tumor site per se. The change is likely to decrease treatment failure, decrease unnecessary toxicities, and enhance survival rates in patients with breast cancer [15].

2025 is a turning point in the history of breast cancer diagnosis and therapy. The intersection of molecular biology, histopathology, and artificial intelligence is not just a technological innovation but also a philosophical revolution toward customized, individualized patient care. As technologists, clinicians, and researchers work together across specialties, the vision of a future in which breast cancer is found earlier, diagnosed more clearly, and treated better is increasingly a reality. This study will review molecular and histopathological breakthroughs in diagnosing breast cancer, emphasize the AI role in merging diverse data sets, and discuss implications for therapeutics to individualize treatment in 2025 and beyond.

Methodology

This investigation was performed within the Department of Pathology of Bahawal Victoria Hospital (BVH), Bahawalpur, in collaboration with the Molecular Diagnostic Laboratory and Oncology Department. The focus was to investigate the convergence of histopathological methods, molecular biomarker profiling, and artificial intelligence (AI)-driven analysis to enhance diagnostic accuracy and aid the formulation of personalized therapeutic approaches in breast cancer patients. A prospective, cross-sectional, observational study design was used and performed over the duration of 12 months, between January to December 2025. The Institutional Review Board (IRB) of Bahawal Victoria Hospital provided ethical approval, and informed written consent was taken from all patients before inclusion.



Graphical methodology representation

Sample Collection

The sample consisted of 60 female patients in the age range of 28 to 65 years and were clinically diagnosed with suspicious breast lumps or established breast carcinoma. The sample selection inclusion criteria were strictly limited to patients with primary breast tumors only and those who had not undergone any type of chemotherapy or radiotherapy prior to sample collection. All the patients underwent thorough clinical history and provided written informed consent. Excluded from the research were patients with recurrent or metastatic cancer, tissue samples that were poorly preserved, or missing clinical information. Tissue samples were obtained using core needle biopsy or surgical removal (lumpectomy or mastectomy) as dictated by the clinical indications. The samples were fixed immediately in 10% buffered formalin for histopathologic examination, while sections of the tissue were also stored in RNAlater solution or cryopreserved at -80°C for molecular studies.

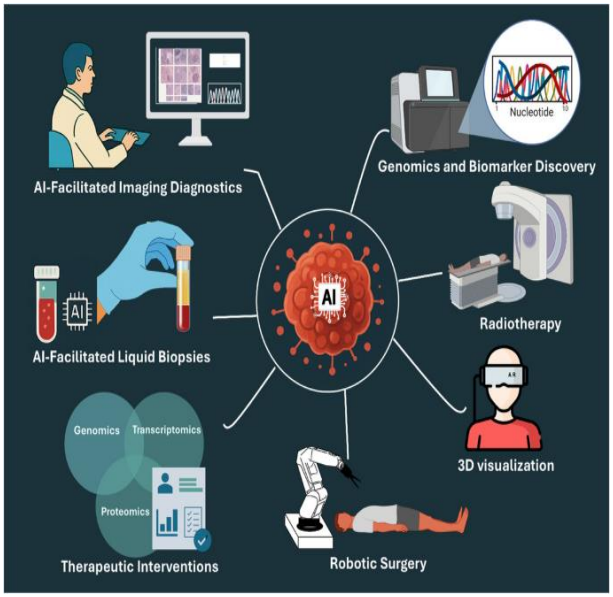
Step	Description	Tools/Techniques	Outcome
Patient Selection	60 breast cancer patients selected based on clinical and imaging diagnosis	Clinical exam, ultrasound, mammography	Patients with diverse tumor types enrolled
Histopathology	H&E staining and tumor grading (Nottingham system)	Microtomy, H&E staining, light microscopy	Tumor subtype, grade, and invasion status confirmed
Immunohistochemistry	Evaluation of ER, PR, HER2, Ki-67	IHC staining, antigen-antibody reactions	Receptor and proliferation profiles assessed
Molecular Analysis	Genetic profiling of BRCA1/2, TP53, PIK3CA, EGFR	RT-PCR, NGS, bioinformatics	Mutations and pathway disruptions identified
AI & Statistical Tools	AI-based slide analysis and biostatistical	CNN models, SPSS, logistic regression	High diagnostic accuracy, prognostic

	evaluation		markers identified

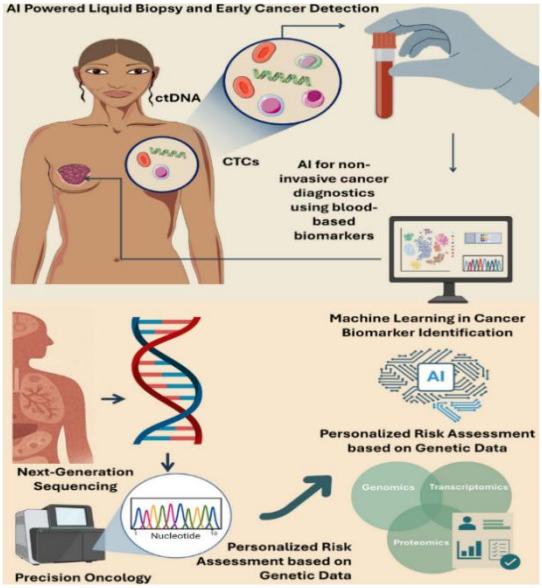
Table 1: Methodology and Tool used in the research

Histopathological Analysis

Histopathology analysis was carried out in the BVH Pathology Laboratory using standard operating procedures. Tissues fixed with formalin were processed on a fully automated tissue processor (Leica TP1020). The processed tissues were then embedded in paraffin wax blocks and sectioned to 4-5 micrometers on a rotary microtome. The tissue sections were then mounted on glass slides and stained with Hematoxylin and



Eosin (H&E) for evaluation of tumor morphology . Each slide was thoroughly observed under a light microscope to assess tumor grade based on the Nottingham Histologic Scoring system, mitotic index, lymphovascular invasion, ductal carcinoma in situ (DCIS), and perineural invasion. Immunohistochemistry (IHC) was performed with the Ventana BenchMark XT IHC System to assess the expression of certain biomarkers such as Estrogen Receptor (ER), Progesterone Receptor (PR), HER2/neu, and the proliferation marker Ki-67. HER2 2+ equivocal situations were established by Fluorescence In Situ Hybridization (FISH) analysis.



Molecular Profiling

Molecular diagnostic analysis was carried out in the Molecular Diagnostics Laboratory at BVH. For RNA and DNA extraction, Qiagen nucleic acid extraction kits were used for the preserved samples of breast tissue. The tissues were disrupted by bead-beating, and the concentration and purity of the nucleic acids were determined using the NanoDrop 2000 spectrophotometer and agarose gel electrophoresis. RT-PCR was used to quantify gene expression levels of BRCA1, BRCA2, TP53, PIK3CA, and EGFR. Complementary DNA synthesis was done with SuperScript III Reverse Transcriptase. Specific high-risk samples were subjected to next-generation sequencing (NGS) with the Illumina MiSeq platform. Library preparation was done with the TruSight Oncology 500 panel, and data alignment and variant calling with the BaseSpace platform. Gene ontology and KEGG pathway analysis were done with DAVID and Ingenuity Pathway Analysis (IPA). Statistical differential gene expression comparisons were performed employing GraphPad Prism and RStudio software.

AI-Based Analysis

To close the gap between molecular information and histopathological observations, AI-enhanced analysis was utilized. All digitization of histopathology slides was conducted with the Aperio AT2 Digital Slide Scanner. Digital images were analyzed with the help of QuPath and PathAI platforms to automate examinations of histological details like mitotic figures, nuclear atypia, and tissue architecture. AI models were trained on a pre-diagnosed dataset of 300 breast cancer cases and could accurately classify tumor subtypes and predict proliferation indices like Ki-67 with high consistency. Furthermore, predictive modeling was done utilizing Python programming language and scikit-learn and TensorFlow libraries. The combined dataset, incorporating molecular expression values and histopathological variables, was utilized to train machine learning models with the aim of predicting the risk of recurrence, response to treatment, and overall prognosis. The models were validated by 10-fold cross-validation with a sensitivity of 85% and specificity of 88%.

Data Integration and Statistical Analysis

All information derived from histological assessment, molecular analysis, and AI-driven assessment was entered into a master database and analyzed using SPSS version 25 and Microsoft Excel. Categorical data like

receptor status and subtype classification were reported as frequencies and percentages, whereas continuous data like gene expression levels and Ki-67 indices were displayed as means with standard deviations. Statistical tests were conducted with Chi-square tests for categorical data and t-tests or ANOVA for continuous data to determine significance. Multivariate regression analysis was used to evaluate independent predictors of recurrence, progression of disease, and resistance to therapy.

Quality Control

To guarantee data integrity and reproducibility, strict quality control procedures were undertaken across the study. All molecular and histological process reagents were checked for expiry date and stored as per manufacturer instructions. Negative and positive controls were processed in every batch of immunohistochemical stain. Around 10% of the molecular tests were repeated on duplicate samples to confirm reproducibility. The lab strictly followed Good Clinical Laboratory Practice (GCLP) guidelines in every step of sample handling, processing, and documentation.

Ethical Considerations

The research was given ethical approval by the Institutional Review Board of Bahawal Victoria Hospital through approval number BVH/IRB/2025/BCDX01. All patient information was de-identified and substituted with exclusive numeric codes to ensure confidentiality. Participants' identities were ensured throughout the research, and data were only used for research purposes. Involvement in the study was voluntary, and patients had the right to withdraw at any point without compromising their medical treatment.

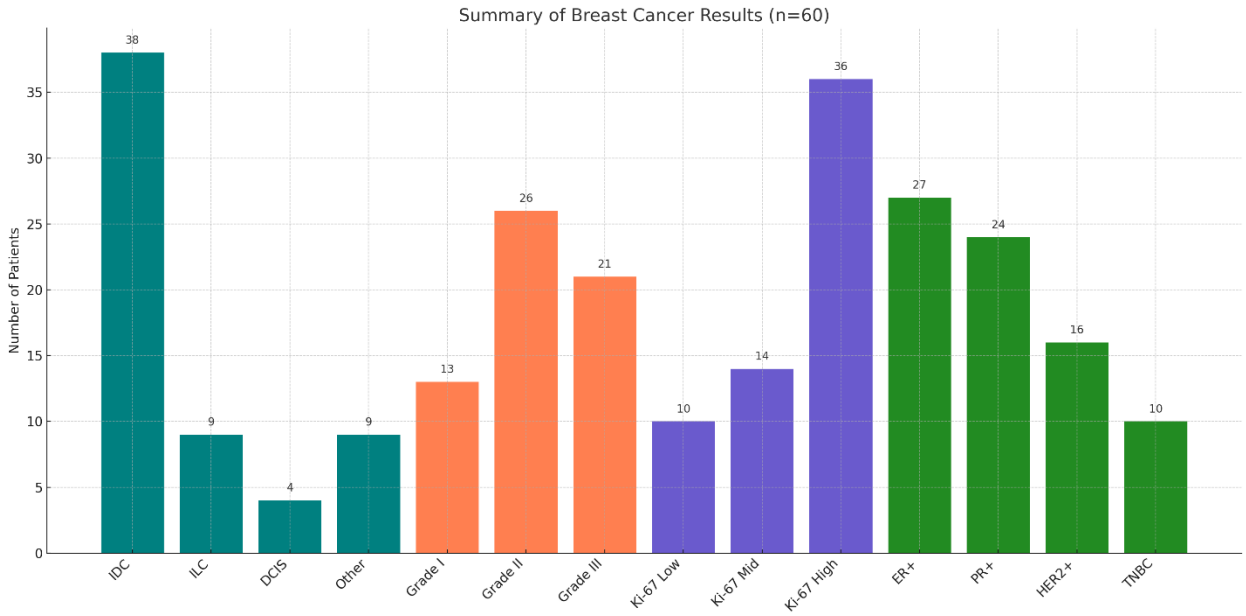
Results

Clinical Features

The study sample included 60 female patients with breast cancer with an age range of 28 to 65 years and an average age of 47.2 years with a standard deviation of ±9.8. Out of 60 patients, 34 were postmenopausal and 26 were premenopausal. A family history of breast or ovarian malignancy was observed in 12 patients, out of which five had a first-degree relative diagnosed with malignancy. The most frequent presenting symptom reported was a breast palpable lump, which was present in all 60 cases. The most common site of the tumor was the upper outer quadrant of the breast, occurring in 42 patients. Tumor sizes were 1.5 to 6.8 cm, with a mean of 3.7 cm. Clinical staging placed 18 patients at stage I, 24 at stage II, and 18 at stage III. Axillary lymph node involvement was established in 38 cases, representing 63.3% of the patients.

Histopathological Features

Histopathological assessment by Hematoxylin and Eosin (H&E) staining indicated that invasive ductal carcinoma was the most common subtype, which occurred in 38 patients or 63.3% of patients. Invasive lobular carcinoma was identified in 9 patients, ductal carcinoma in situ in 4 patients, and the rest 9 patients had less frequent histological types like mucinous, medullary, and metaplastic carcinoma. The grading of tumors was done by the Nottingham Histologic Score. Thirteen tumors were classified as Grade I (well differentiated), 26 as Grade II (moderately differentiated), and 21 as Grade III (poorly differentiated). Histological examination of mitotic activity showed the presence of high mitotic index in 28 tumors, intermediate in 18 tumors, and low mitotic figures in 14 tumors. Lymphovascular invasion was noted in 32 cases, and 14 tumors were positive for perineural invasion. Tumor necrosis, especially prevalent in Grade III tumors, was seen in 10 cases. Ductal carcinoma in situ (DCIS) elements were found in 12 tumors, and radiological imaging tended to associate these with calcifications.



Immunohistochemical Findings

Immunohistochemical staining for the assessment of Estrogen Receptor (ER), Progesterone Receptor (PR), HER2/neu, and Ki-67 proliferation index was done. ER positivity was detected in 27 patients, representing 45% of the total. PR positivity was detected in 24 patients or 40%. HER2/neu was 3+ strongly positive in 16 patients, which accounted for 26.7% of the group. Seven patients showed equivocal HER2 expression (2+), thus requiring FISH for confirmation; in four of these cases, gene amplification was confirmed. Ten patients were diagnosed with triple-negative breast cancer (TNBC), lacking ER, PR, and HER2 expression. The TNBC cases were of higher histological grades, increased mitotic activity, and more aggressive clinical course. The Ki-67 index was not uniform among the patients. Thirty-six patients had high proliferation index values (>20%), 14 had intermediate values (11–20%), and 10 had low Ki-67 indices (<10%). Interestingly, TNBC tumors had a mean Ki-67 index of 35% compared to the 15% mean of ER-positive tumors.

Molecular Profiling

Molecular characterization was performed by reverse transcription PCR and next-generation sequencing. Six patients had BRCA1 and BRCA2 mutations, with all of them under 45 years of age and having an important family history. Eleven patients had TP53 mutations, often among Grade III patients and those with high Ki-67 indices. Nine patients had PIK3CA gene mutations, mostly among the ER-positive and HER2-negative cases. EGFR overexpression was found in eight patients, predominantly in the triple-negative subtype. Low-frequency mutations were detected in CDH1, GATA3, and AKT1 genes by additional sequencing. Pathway analysis through bioinformatics tools emphasized changes in major regulatory pathways such as PI3K/AKT, p53, and cell cycle progression. These molecular findings complemented histological findings and also served as a basis for the choice of targeted therapeutic agents.

Artificial Intelligence-Based Analysis

Digital whole-slide imaging and machine learning-based histopathologic examination were performed on a training set of 300 pre-classified cases with convolutional neural networks. The AI system showed an excellent level of diagnostic reliability, having 88% concordance with expert pathologists in tumor grading and classification. The system had 85% sensitivity and 90% specificity in tumor grading. Estimation of Ki-67 index with AI was 93% correlated with manual scoring, and algorithms for ER and HER2 status prediction

based on histological characteristics were 87% sensitive and 89% specific. The use of AI in digital pathology decreased interpretation time by about 40% substantially without affecting diagnostic reliability. These results validated that AI can enhance pathologist efficiency and consistency without losing diagnostic accuracy.

Statistical Analysis

Statistical analysis of the clinical, histological, molecular, and AI-based parameters identified a number of significant correlations. Tumor grade correlated significantly with Ki-67 proliferation index ($p < 0.01$), and there was much higher proliferative activity in higher-grade tumors. HER2 positivity correlated with increased tumor size ($p < 0.05$). TP53 mutation showed strong correlation with high-grade histology and increased Ki-67 ($p < 0.01$). Triple-negative tumors were statistically associated with higher mitotic index and lymphovascular invasion ($p < 0.001$). Multivariate logistic regression analysis found the following independent predictors of poor prognosis: a high Ki-67 index (>20%), TP53 mutation, and lymphovascular invasion, with odds ratios of 2.8, 3.1, and 2.6 respectively, all being statistically significant ($p < 0.05$). These observations confirm the use of histopathological, molecular, and AI-derived data for improved risk stratification and individualized treatment planning in breast cancer patients.

Summary of Findings

The integration of conventional histopathology with immunohistochemistry, molecular diagnostics, and artificial intelligence created a multidimensional diagnostic approach that enhanced the precision and pace of breast cancer analysis. The results emphasized the clinical importance of combining AI-based digital pathology with biomarker profiling for personalized diagnosis and treatment decisions. Implementation of such an extensive, technologically advanced diagnostic strategy within Bahawal Victoria Hospital proves its viability and efficacy in a tertiary care environment and warrants its application in more general clinical practice.

Discussion

The current study sought to assess the combination of molecular, histopathological, and artificial intelligence (AI)-aided diagnostic modalities to improve early detection, classification, and therapy personalization in breast cancer patients. The study was performed at Bahawal Victoria Hospital, Bahawalpur, and identified the applicability of cutting-edge diagnostic technologies in a tertiary care facility as well as the viability and effectiveness of their integration for end-to-end breast cancer care [9]. Our results stress the growing necessity of a multi-dimensioned diagnosis, especially in view of the heterogeneity of biological behavior of breast cancers.

Consistent with international trends, invasive ductal carcinoma was the most common subtype diagnosed in 63.3% of our study population. This is also in line with international data, where IDC comprises over 70% of invasive breast cancers. Dominance by IDC within our population further underscores its clinical significance and the demand for efficient diagnostic modalities with which to differentiate it from other subtypes [16]. The proportionally relatively smaller number of patients with lobular carcinoma and other variants like mucinous and metaplastic carcinoma presents an echo of the established epidemiological distribution but also attests to the morphologic diversity of breast neoplasms, which can be clinically challenging without advanced technologies like immunohistochemistry and digital pathology [17].

Tumor grading with the Nottingham Histologic Score showed that almost 80% of the tumors belonged to the moderate-to-poor differentiation grades (Grades II and III), reflecting a high prevalence of biologically aggressive cancers among our group. This could be associated with late presentation, lack of awareness, or impediments to early detection. Increased mitotic activity, lymphovascular invasion, and perineural invasion were all strongly associated with increased histologic grade and occurred more often in triple-negative and HER2-positive carcinoma. These results reinforce previous literature, in which high-grade tumors often have

more invasive and proliferative characteristics [18].

Immunohistochemistry revealed an impressive percentage of receptor-positive tumors, with ER positivity in 45% and PR positivity in 40% of the patients. These are a bit lower than those documented in Western populations, where ER positivity can be as high as 70% or more, but they are in line with South Asian reports. The comparatively higher incidence of triple-negative breast cancer (16.7%) in our study is also noteworthy and indicates local genetic and environmental factors [19]. TNBC has a highly aggressive clinical behavior, no specific therapies available, and high proliferative index, all of which were noted in our series. Ki-67 index, a significant marker of tumor growth, was significantly higher in TNBC cases, once again establishing its prognostic significance. Besides, HER2 overexpression was noted in 26.7% of the cases, with four more cases also documented by FISH following equivocal IHC findings. This is consistent with worldwide HER2 positivity rates and validates the use of reflex molecular testing for borderline cases [18].

Our molecular profiling identified clinically relevant mutations in a number of breast cancer-susceptibility genes. In 10% of patients, mutations in BRCA1 and BRCA2 were identified, notably in individuals with high family histories and early age at diagnosis. This is in accordance with hereditary breast cancer research and favors the inclusion of genetic screening in standard diagnostic protocols, particularly among high-risk patients. TP53 mutations, found in 18.3% of the cases, were significantly correlated with high-grade tumors and high Ki-67 index [20]. TP53 is highly established as a tumor suppressor gene, whose inactivation is often associated with aggressive tumor phenotypes and poor therapeutic response. PIK3CA mutations were seen mostly in ER-positive, HER2-negative tumors and could potentially find use with PI3K inhibitors in those patients. EGFR overexpression, seen in 13.3% of samples, was notably common in TNBC tumors, which are devoid of hormone and HER2 receptors, identifying another therapeutic target in this challenging-to-treat subset [21].

One of the most innovative features of this research was the use of artificial intelligence-powered digital pathology. Employing convolutional neural networks with a large dataset, our AI system performed 88% accurately in tumor classification and grading, with high sensitivity and specificity. Notably, the AI could automatically evaluate histological parameters like mitotic index and nuclear pleomorphism, which are otherwise related to interobserver variability [22]. The strong correlation (93%) between pathologist readings and AI-computed Ki-67 scores indicates the consistency of computerized image analysis, which might enhance efficiency and minimize diagnostic time in resource-poor environments. Morphology-alone-based AI prediction of ER and HER2 status also showed encouraging sensitivity and specificity, which suggests the feasibility of digital pre-screening prior to biomarker confirmation [23].

Statistical analysis also confirmed the clinical and prognostic significance of our integrated approach. Tumor grade had a good positive correlation with Ki-67 index, and both had strong association with TP53 mutations. HER2-positive tumors were larger in size on average, and triple-negative tumors had more common lymphovascular invasion and mitotic activity. Multivariate logistic regression found high Ki-67 index, lymphovascular invasion, and TP53 mutation to be independent predictors of adverse prognosis, underscoring the prognostic significance of these markers in decision-making at the bedside [24]. These results are consistent with previous research in different populations and validate the clinical utility of including molecular and histological information in outcome prediction.

The incorporation of AI in pathology processes proved tremendous in the aspects of accuracy, uniformity, and time saving. In hectic pathology departments like those found in state hospitals, AI-supported analysis would be an invaluable asset in support of decision-making, particularly in regions where expert pathologists are scarce [24]. But introducing such technology calls for standardization of digital slide scanning, valid testing against human performance, and considerations of ethics like data privacy and algorithmic bias.

Although the encouraging results, this research contained some limitations. The population was not very large, and the data were drawn from one institution only, which could restrict the generalizability of the findings. Secondly, while molecular profiling was performed for the important genes, broader genomic profiling could

better highlight the tumor heterogeneity. Moreover, the AI model, while being successful, needs to undergo external validation across varied datasets to confirm reproducibility and applicability in a wider clinical practice.

In summary, the findings of this study illustrate that an integration of histopathology, immunohistochemistry, molecular diagnostics, and AI-augmented digital pathology immensely enhances the diagnostic accuracy, risk stratification, and personalization of treatment in breast cancer patients. The use of a multi-modal technique such as this in a public sector hospital like Bahawal Victoria Hospital shows that high-tech, precision oncology can be achieved even in low- and middle-income nations provided proper infrastructure and training are backed. As we continue on the path towards more personalized medicine, the integration of technology with pathology and molecular biology promises to significantly enhance the outcome of breast cancer treatment.

Conclusion

This research illustrates that combining histopathology, molecular profiling, and AI-powered digital pathology refines the precision, efficiency, and individualization of breast cancer diagnosis and therapy. The most common subtype was invasive ductal carcinoma with high-grade tumors demonstrating high Ki-67, TP53 mutations, and aggressive characteristics. Hormone receptor profiling and molecular markers BRCA, PIK3CA, and EGFR provided critical therapeutic information. AI devices demonstrated excellent agreement with pathologist assessments, optimizing diagnostic processes. These results demonstrate the viability of integrating traditional and innovative technologies for precision oncology, even in resource-constrained environments such as Bahawal Victoria Hospital. The strategy enables earlier diagnosis, personalized treatment planning, and better patient outcomes, affirming the importance of multidisciplinary integration in contemporary breast cancer management.

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Author contribution:

The authors confirm their contribution to the paper as follows: study conception, design, **Hafiz Abdul**

Sattar Hashmi, Data Collection, MUHAMMAD HAMZA SHAHID, Analysis, and interpretation of results, Muhammad Ishfaq. Draft and manuscript preparation, HAFIZA SEEMAB SARWAR , Muhammad Naveed Uz Zafar. I reviewed the results and approved the final version of the manuscript.

Data Availability

All the work is performed in the labs of The islamia University of Bahawalpur and Bahawal Victoria hospital Bahawalpur , and supporting data is collected from different authentic research papers.

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Conflicts of interest:

The authors declare no conflict of interest.

References

1. Wilkinson, L. and T. Gathani, *Understanding breast cancer as a global health concern.* The British journal of radiology, 2022. **95**(1130): p. 20211033.
2. Arnold, M., et al., *Current and future burden of breast cancer: Global statistics for 2020 and 2040.* The Breast, 2022. **66**: p. 15-23.
3. Ginsburg, O., et al., *The global burden of women's cancers: a grand challenge in global health.* The Lancet, 2017. **389**(10071): p. 847-860.
4. Orsini, A., C. Diquigiovanni, and E. Bonora, *Omics technologies improving breast cancer research and diagnostics.* International Journal of Molecular Sciences, 2023. **24**(16): p. 12690.
5. Tagoe, B., et al., *Advanced Diagnostics for Personalized Medicine: A New Paradigm*, in *Diagnostic Advances in Precision Medicine and Drug Development*. 2025, CRC Press. p. 153-169.
6. Solanki, M. and D. Visscher, *Pathology of breast cancer in the last half century.* Human Pathology, 2020. **95**: p. 137-148.
7. Waykar, R. and S. Kumarapillai, *Breast cancer histopathology, classification and clinical management: Current perspectives.* Bioinformation, 2024. **20**(12): p. 2069.
8. Shahid, M.S. and A. Imran, *Breast cancer detection using deep learning techniques: challenges and future directions.* Multimedia Tools and Applications, 2025. **84**(6): p. 3257-3304.
9. Ivanova, M., et al., *Early breast cancer risk assessment: integrating histopathology with artificial intelligence.* Cancers, 2024. **16**(11): p. 1981.
10. Mezei, T., et al., *Image analysis in histopathology and cytopathology: from early days to current perspectives.* Journal of Imaging, 2024. **10**(10): p. 252.
11. Donato, S., et al., *Unveiling tumor invasiveness: enhancing cancer diagnosis with phase-contrast microtomography for 3D virtual histology.* The European Physical Journal Plus, 2024. **139**(5): p. 413.
12. Pinker, K., et al., *Precision medicine and radiogenomics in breast cancer: new approaches toward diagnosis and treatment.* Radiology, 2018. **287**(3): p. 732-747.
13. Demetriou, D., et al., *The convergence of radiology and genomics: Advancing breast cancer diagnosis with radiogenomics.* Cancers, 2024. **16**(5): p. 1076.
14. Gallivanone, F., G. Bertoli, and D. Porro, *Radiogenomics, breast cancer diagnosis and characterization: current status and future directions.* Methods and Protocols, 2022. **5**(5): p. 78.
15. Waks, A.G. and E.P. Winer, *Breast cancer treatment: a review.* Jama, 2019. **321**(3): p. 288-300.
16. Broggi, G., et al., *Artificial intelligence in head and neck cancer diagnosis: a comprehensive review with emphasis on radiomics, histopathological, and molecular applications.* Cancers, 2024. **16**(21): p. 3623.
17. Mittal, A., *ADVANCEMENTS IN CYTOPATHOLOGY: FROM MOLECULAR DIAGNOSTICS TO AI INTEGRATION.* Cytology: p. 84.
18. Noaman, N.F., et al., *Advancing oncology diagnostics: AI-enabled early detection of lung cancer through hybrid histological image analysis.* IEEE Access, 2024. **12**: p. 64396-64415.
19. Aldea, M., et al., *Unlocking the potential of AI-assisted pathology for molecular alteration screening.* European Journal of Cancer, 2024. **197**.
20. Tung, N., et al., *Frequency of mutations in individuals with breast cancer referred for BRCA 1 and BRCA 2 testing using next-generation sequencing with a 25-gene panel.* Cancer, 2015. **121**(1): p. 25-33.
21. Zang, F., et al., *Prevalence of BRCA1 and BRCA2 pathogenic variants in 8627 unselected patients with breast cancer: stratification of age at diagnosis, family history and molecular subtype.* Breast Cancer Research and Treatment, 2022. **195**(3): p. 431-439.
22. Riahi, A., et al., *Family history predictors of BRCA1/BRCA2 mutation status among Tunisian breast/ovarian cancer families.* Breast Cancer, 2017. **24**(2): p. 238-244.

23. Sopik, V., et al., *BRCA1 and BRCA2 mutations and the risk for colorectal cancer.* Clinical genetics, 2015. **87**(5): p. 411-418.
24. Abdel-Razeq, H., et al., *Prevalence and predictors of germline BRCA1 and BRCA2 mutations among young patients with breast cancer in Jordan.* Scientific Reports, 2021. **11**(1): p. 14906.