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HLA-B expression in breast cancer patients in southwestern Nigeria: A descriptive study

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Abstract

Introduction: This study aimed to evaluate human leukocyte antigen B (HLA-B) expression in breast cancer tissues from two tertiary health institutions in southwestern Nigeria and explore its relationship with age at diagnosis.
Methods: A cross-sectional study was conducted to investigate the immunohistochemical expression of HLA-B in the histologic tissues of breast cancer patients at the Obafemi Awolowo University Teaching Hospitals Complex, which was diagnosed between January 1, 2022, and December 31, 2022. The tissue samples were formalin fixed and paraffin embedded (FFPE) to preserve their morphology and antigenicity. We used an anti-HLA-B antibody (Clone PAS-35345) from Thermo Fisher Scientific as the primary antibody.
Results: Thirty-eight cases of breast cancer were evaluated during the study period. Half of the breast cancer patients expressed little or no HLA-B protein. High and moderate expression was observed in only 7 (18.4%) and 10 (26.3%) of the patients, respectively. A comparison of the expression of HLA-B across the different age groups revealed no statistically significant differences between the age groups ($p = 0.898$).
Conclusion: Our study revealed that over 40% of breast cancer patients exhibited moderate to high HLA-B expression, suggesting that a significant proportion of these cancers may be amenable to immunotherapy. There was no significant variation in the degree of expression of HLA-B across the various age groups at presentation. This could also mean that HLA-B expression is not particularly related to early-onset breast cancer.

Keywords: HLA-B Antigens, Immunohistochemistry, Breast cancer, Immunity

Introduction

The human leukocyte antigen (HLA) complex plays a pivotal role in the immune system, facilitating the recognition and elimination of pathogens and malignant cells. HLA-B, a key component of the class I major histocompatibility complex (MHC), is crucial for presenting antigens to cytotoxic CD8+ lymphocytes, thereby triggering an immune response. The expression of HLA in tumour cells has been linked to improved prognosis, as it enhances

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lymphocytic infiltration and activates immune-mediated tumour control.[1,2]

Breast cancer, a leading cause of cancer-related mortality worldwide, is associated with significant disparities in outcomes between African and Western populations. Nigerian and African breast cancer patients often present at advanced stages and display aggressive immunophenotypes, rendering conventional treatments (surgery, chemotherapy, and hormonal therapy) less effective. The morbidity and mortality rates are so high that the incidence of breast cancer is projected to reverse the increase in maternal mortality in the coming years.[3,4] Aggressive breast cancer is also common in people of African descent living in other parts of the world.[5–7] This highlights the need for alternative therapeutic approaches, such as immunotherapy.

HLA has several genetic polymorphisms associated with susceptibility to breast cancer. This can occur through its effect on immune activation or its relationship with various viruses.[8,9] Some researchers have linked breast cancer to several viral infections.

Quantitative polymerase chain reaction (qPCR) is a suitable method for assessing HLA expression. However, several studies have demonstrated that immunohistochemistry (IHC) is also a reliable approach for evaluating HLA expression.[10] Compared with qPCR, IHC offers several advantages, including lower costs and reduced complexity. Additionally, IHC allows for the assessment of HLA-B expression in both whole-tissue sections and tissue microarrays, providing flexibility in experimental design.

HLA-B expression has been investigated in various tumors, revealing its potential as a prognostic biomarker and predictor of treatment outcomes. However, data on HLA-B expression in breast cancer tissues from Nigerian patients are scarce. Our study aims to address this knowledge gap by evaluating HLA-B expression in breast cancer tissues from our center and exploring its relationship with age at diagnosis. This research provides valuable insights into the immune landscape of breast cancer in our population, serving as a foundation for future studies and informing future management strategies, including immunotherapy.

Methods

A cross-sectional study was conducted to investigate the expression of human leukocyte antigen B (HLA-B) in the histologic tissues of breast cancer patients at Obafemi Awolowo University Teaching Hospital and two other tertiary health institutions in southwestern Nigeria.

The study population consisted of patients diagnosed with breast cancer at Obafemi Awolowo University Teaching Hospital between January 1, 2022, and December 31, 2022.

Inclusion criteria included patients who had a histologically confirmed breast cancer diagnosis; underwent mastectomy, lumpectomy, or biopsy; had available formalin-fixed, paraffin-embedded (FFPE) tissue samples; and provided informed consent for the use of their tissue samples in research.

Exclusion criteria were patients with insufficient tissue samples for analysis; had prior radiotherapy, chemotherapy, or hormonal therapy for breast cancer; had inadequate clinical or pathological data; or declined informed consent for the study.

Breast cancer tissue samples were collected from patients with various histological subtypes. The samples used consisted of Trucut biopsy samples as well as mastectomy samples. The tissue samples were formalin fixed and paraffin embedded (FFPE) to preserve their morphology and antigenicity. The tissues were processed via tissue microarray analysis with a large bore needle.

The samples were stained with an anti-HLA-B antibody (Clone PAS-35345) from Thermo Fisher Scientific as the primary antibody. Positive and negative external and internal controls were employed. All the antibodies, retrieval buffers, and detection kits were obtained from Thermo Fisher Scientific to ensure consistency. Automated IHC staining was performed onboard the antigen retrieval system via pressure cooking. The tissue sections were incubated with the HLA-B antibody at a dilution of 1:40. After staining, the slides were dehydrated, cleared, and mounted for microscopic examination.

The stained slides were examined under a microscope, with a focus on the pattern and intensity of HLA-B expression. The degree of HLA-B expression was assessed on the basis of the intensity of the immunostaining and the percentage of tumor cells stained. Both cytoplasmic and membranous staining were evaluated. The nuclear or cytoplasmic staining intensity (0–3, where 0 is no staining, 1 is weak, 2 is moderate, and 3 is strong) was multiplied by the percentage of positively stained cells (0–3, where 0 is ≤ 5%, 1 is 6–25% positive cells, 2 is 26–50% positive cells, and 3 is ≥51% positive cells) to create a semiquantitative IHC score. Nine is the maximum achievable IHC score. A score of 0

indicates no expression; a score of less than or equal to 3 indicates low expression; a score between 3 and 4.25 indicates moderate expression; and a score greater than 4.25 indicates high expression.

Data analysis

The association between HLA-B expression and patient age was analysed via simple descriptive statistics in SPSS 20. Results were presented in tables and charts.

Results

Thirty-eight cases of breast cancer were evaluated during the study period. These patients had adequate remaining tissue after trimming and processing via the tissue microarray technique. The patients had infiltrating ductal carcinoma (not otherwise specified). The age group with the most frequent cases was the 51-60 years , which accounted for 34.2% of all cases. Ages 31-40 accounted for the minimum proportion of cases (10.5%). The average age was 54.92 ± 12.30 years. Figure 1 shows the age distribution of the patients.

Figure 1 above shows an approximately normal distribution, with the age group 51--60 years accounting for most of the breast cancer cases.

Half of the breast cancer patients expressed little or no HLA-B protein. Twelve (31.6%) breast cancers had no expression of HLA-B, whereas 9 (23.7%) cancers had low expression of the marker. High and moderate expression was observed in only 7 (18.4%) and 10 (26.3%) patients respectively. Figures 2 and table 1 show the relative frequencies of the pattern of HLA-B expression and how it relates to the various age groups. Figures 3, 4 and 5 show photomicrographs of

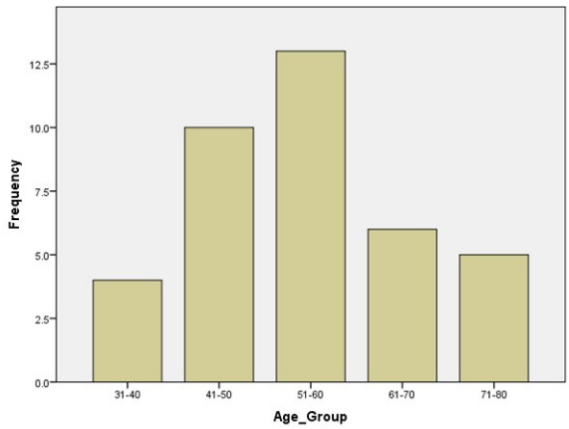

Figure 1: Age distribution of the breast cancer patients

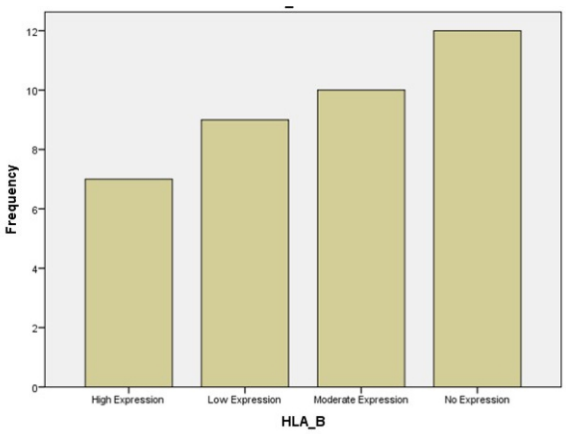

Figure 2: Bar chart showing the frequencies of the various categories of HLA-B expression

Table 1: Table showing the relative frequencies of the various categories of HLA-B expression across the age groups

	HLA-B expression				
Age group (years)	High	Moderate	Low	None	Total
31-40	1 (25%)	0 (0%)	1 (25%)	2 (50%)	4 (100%)
41-50	2 (20%)	3 (30%)	1 (10%)	4 (40%)	10 (100%)
51-60	2 (15%)	3 (23.1%)	5 (38.5%)	3 (23.1%)	13 (100%)
61-70	1 (17%)	3 (50%)	1 (16.7%)	1 (16.7%)	6 (100%)
71-80	1 (20%)	1 (20%)	1 (20.0%)	2 (40%)	5 (100%)
Total	7 (18%)	10 (26.3%)	9 (23.7%)	12 (31.6%)	38 (100%)

P=0.898

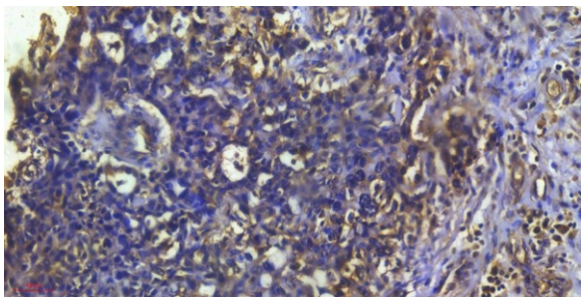

Figure 3: Strong HLA_B expression by malignant cells

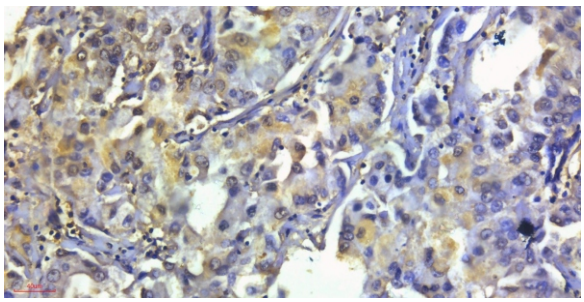

Figure 4: Moderate HLA_B expression by malignant cells

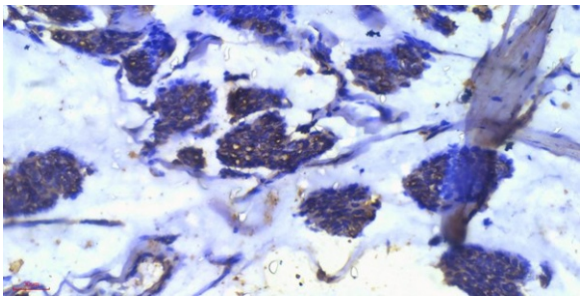

Figure 5: Moderate HLA_B expression by malignant cells in an infiltrating ductal carcinoma with a mucinous pattern

HLA-B expression at various foci in the tumors studied.

Figure 2 shows that most breast cancer tissues did not express HLA-B. However, few tissues presented high HLA-B expression.

A comparison of the expression of HLA-B across the different age groups revealed no statistically significant differences between the age groups ($p = 0.898$). This is shown in table 1.

Discussion

The small number of cases recorded in this study was the result of tissue loss during processing via the tissue microarray technique. TMA as a research tool has not been embraced by researchers in Nigeria, partly as a result of a lack of equipment and technical knowledge.

Our study revealed that over 40% of breast cancer patients exhibited moderate to high HLA-B expression, suggesting that a significant proportion of these cancers may be amenable to immunotherapy. This finding is particularly important in the Nigerian context, where many breast cancers are aggressive, hormone receptor-negative, and present at advanced stages, limiting treatment options.[3,4,6,7]

The absence of routine immunohistochemistry in many cases due to resource constraints highlights the need for alternative diagnostic approaches. It is necessary to evaluate the proportion of patients with moderate to high HLA-B expression among the various immunophenotypes. HLA-B expression in triple-negative breast cancers will be of particular interest. This is because these groups of patients who present at late stages have very few treatment options available. Evaluating HLA-B expression in various immunophenotypes, particularly triple-negative breast cancers, is crucial for identifying potential targets for immunotherapy.[11–13]

While our study focused on infiltrating ductal carcinoma, investigating HLA-B expression in other histologic subtypes may reveal correlations between histologic type and HLA-B expression. This could enable the prediction of HLA-B expression from hematoxylin and eosin-stained tissue sections, which is valuable in resource-limited settings.

This study did not reveal any significant variation in the degree of expression of HLA-B across the various ages at presentation. This finding might suggest that HLA-B expression by breast cancer cells does not have a significant easily observable effect on the rate of development and hence the presentation of the tumor. This could also mean that it is not particularly related to early-onset breast cancer. In our study, the early-stage breast cancer patients in the 31–40 years age group did not have a higher or lower degree of HLA-B expression than those in the other age groups did. To our knowledge, no study has evaluated the relationship between age of breast cancer onset and HLA-B expression.

It is important to check for various HLA-B subtypes to determine whether these subtypes are associated with increased risk of breast cancer. This is necessary as it can be used to predict individuals who are at greater risk and may then require more frequent screening procedures to enable physicians to detect new lesions very early.

The majority of our patients presented little or no HLA-B expression, indicating potential immune evasion and tumor progression. Several researchers have suggested an improved prognosis in breast cancers expressing HLAs. This is attributed to a heightened immune response against cancer cells that express HLAs, potentially leading to increased relapse-free and overall survival.[12,13] This finding may contribute to the poor prognosis of breast cancer patients in Nigeria. A larger, more statistically robust study is needed to definitively link HLA-B expression to the disease-free interval or overall survival.

Longitudinal studies of HLA-B expression in relation to tumor progression and metastasis could elucidate disease mechanisms and identify potential therapeutic targets. Additionally, investigating the density and composition of tumor-infiltrating lymphocytes in relation to HLA-B expression may provide further insights into immune responses in breast cancer.

Conclusion

Our study revealed that over 40% of breast cancer patients exhibited moderate to high HLA-B expression, suggesting that a significant proportion of these cancers may be amenable to immunotherapy.

Compared with other age groups, early breast cancer patients in the 31–40 years age group do not have a higher or lower degree of HLA-B expression. There was no significant variation in the degree of expression of HLA-B across the various ages at presentation. This could also mean that HLA-B is not particularly related to early-onset breast cancer.

Longitudinal studies of HLA-B expression in relation to tumor progression and metastasis could elucidate disease mechanisms and identify potential therapeutic targets.

Declarations

Ethics approval and consent to participate: We obtained Informed Consent from the patients in accordance with the guidelines of the Ethical and Research Committee of the Obafemi Awolowo University Teaching Hospitals Complex. The study was approved by the Ethical and Research Committee of the Obafemi Awolowo University Teaching Hospitals Complex. (Ethics approval No NHREC/01/01/2007-24/10/2019)

Competing interests: We declare no conflicts of interest.

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Authors' contributions: KAA, GOO, and NOA initiated the work. OO contributed samples to the study. AAA, AOA and KAA did the laboratory work. OOO and LAB carried out the statistical analysis and produced the charts. OOO and KAA obtained the photomicrographs and wrote the manuscript. OOO, NOA, OO, GOO, AAA, AOA, KAA, LAB, RAB and DAO read and approved the final write-up. KAA, GOO, and NOA supervised the work.

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