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In SITU HYBRIDIZATION and immunohistochemical technique for Epstein-*Barr* Virus (EBV) detection in Misan province breast cancer women

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Abstract

Breast cancer continues to be the most common malignancy and the primary cause of cancer-related mortality among women globally. Although conventional risk factors inadequately account for a considerable number of cases, recent findings associate viral infections, particularly Epstein-Barr Virus (EBV), with the onset and advancement of breast cancer. This research examines the existence and function of EBV in breast cancer among women in Misan Province, Iraq. Immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH) were employed to analyze 70 formalin-fixed, paraffin-embedded breast tissue blocks, comprising 45 malignant and 25 non-malignant samples. The results indicated a prevalent incidence of invasive ductal carcinoma (IDC) (93.3%) among malignant cases, with EBV EBER RNA signals identified in 62% of breast cancer samples against 10% in controls, demonstrating statistically significant differences ($p < 0.04$). Nuclear brown staining indicative of EBV was identified in 68% of cancer tissues using IHC. The data indicate a possible correlation between EBV infection and breast cancer, underscoring the necessity for additional research into its oncogenic mechanisms and implications for preventative and treatment approaches.

Keywords: Breast cancer, EBV DNA, CISH, IHC

Introduction

Breast cancer is the most common cancer in women and the leading cause of cancer-related deaths (1). Global data predict that incidence and mortality will continue to rise (2). Early diagnosis of breast cancer is essential for effective therapy and survival (3). Age, early menarche, late menopause, hormone replacement therapy, and positive family history are risk factors for this cancer (4). Up to 80% of breast cancer patients have no identified risk factors, making the disease sporadic (5). Thus, most breast cancer patients have an unknown etiology, making it crucial to investigate other risk factors. Recent studies have linked infectious pathogens to breast cancer carcinogenesis and onco-modulation (6,7) The earliest evidence that viral infections may cause some human malignancies was in 1964 (8). Since then, several pathways have been connected to virus-induced tumors. Seven human viruses are oncogenic: HPV, HBV, HCV, EBV, KSHV, MCPyV, and HTLV-1 (9). Human and animal oncogenic viruses such as MMTV, BLV, HPVs, and EBV may specifically cause breast cancer (10). Virus-induced malignancies offer prophylactic, diagnostics, and

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treatment prospects due to the tremendous efficacy of vaccines and antivirals in lowering viral tumors (11). The widespread and common human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) have garnered attention in recent decades as reports link HCMV to a range of cancers, including breast cancer. However, the full overview of Breast cancer in Misan Province is not fully understood. Therefore, the main focus of the current study is to evaluate the prevalence of breast cancer among Misan women and detect EBV in cancer blocks.

Study objectives:

To establish if EBV plays a role in breast cancer progression by detecting EBV DNA in malignant breast tissues and comparing the results with normal breast tissues using immunohistochemistry and chromogenic in situ hybridization. In addition, Understanding the prevalence of age groups of Iraqi women with breast cancer could lead to novel preventative or treatment measures.

Patients and method

This study examined the incidence of Epstein-Barr Virus EBV in breast cancer in southern Iraqi women using immunohistochemistry and in situ hybridization. It involved 70 selected archival formalin-fixed, paraffin-embedded breast tissue blocks with improved fixation and processing including the following groups: the data was analyzed by Statistical Package for Social Sciences (SPSS), version twenty-five and the Excel program was also used for graphical figures, and charts.

Results

1. Classification of all block samples:

The present study comprised seventy (70) blocks of breast tissue, of which fifty (45) blocks (64.3%) were diagnosed with breast carcinoma, specifically invasive ductal carcinoma, and invasive lobular carcinoma, serving as the study cases. The remaining thirty (25) blocks (35.7%) contained non-malignant breast tissues, utilized as the control group. The case and control groups were analyzed for the detection of Epstein-Barr Virus EBV virus in populations of Iraqi women (Misan province/ Iraq) utilizing immunohistochemistry (IHC) and in situ hybridization techniques (CISH). The frequency distribution results for breast carcinoma cases indicated that the predominant histological type was invasive ductal carcinoma (IDC), comprising 42 cases (93.3%), followed by invasive lobular carcinoma (ILC) with 4 cases (6.6%). In contrast, other types of breast carcinoma were not identified. The histological grades of breast cancer indicated that grade 2 differentiated breast cancer was diagnosed in 25 out of 45 cases, constituting 55.5% of the total. In comparison, grade 3 differentiated breast cancer was identified in 12 out of 45 cases, representing 30%, and grade 1 differentiated breast cancer was detected in 12 out of 45 cases, accounting for 26.6%. The summary of all the above results has been displayed in Table (1).

Table (1): the summary for all the above results has been displayed in

Breast tissues distributed	Histological type	Histological grades (50)	
(45) blocks (64.3%)	invasive ductal carcinoma (IDC) 42, (93.3%)	Grad1 (8)	IDC 7
			ILC 1
(25) blocks (35.7%)	invasive lobular carcinoma (ILC) 3, (6.6%)	Grad2 (25)	IDC 24
			ILC 1
	Other types of breast carcinoma, not identified	Grad3 (12)	IDC 11
			ILC 1

2- Evaluation of EBV Signals between study groups

The EBV DNA genome was identified in study groups via CISH and IHC techniques. The signals were identified as nuclear brown staining of tumor cells, indicating a positive result for IHC, while a vivid red pattern discoloration at the site of complementary sequences in the nuclei of infected cells was observed for the CISH approach. EBV EBER RNA signals were identified in 31 (68.8%) of the breast cancer cases, whereas 14 (31.1%) exhibited negative results. In the control group, EBV EBER RNA signals were identified in 3 (12%) of the subjects, whereas 22 (88%) exhibited negative results. Notable variations (P<0.04) were seen between the study groups concerning EBV EBER RNA positive findings. The study indicated that immunohistochemistry revealed nuclear brown staining in 34 (68%) of the breast cancer patients, whereas 16 (32%) exhibited negative results. In the control group, EBV nuclear brown staining of tumor cells was observed in 3 (10%) of the subjects, whereas 27 (90%) exhibited negative results. Significant variations (P<0.03) were seen between the research groups for the nuclear brown staining of tumor cell signals.

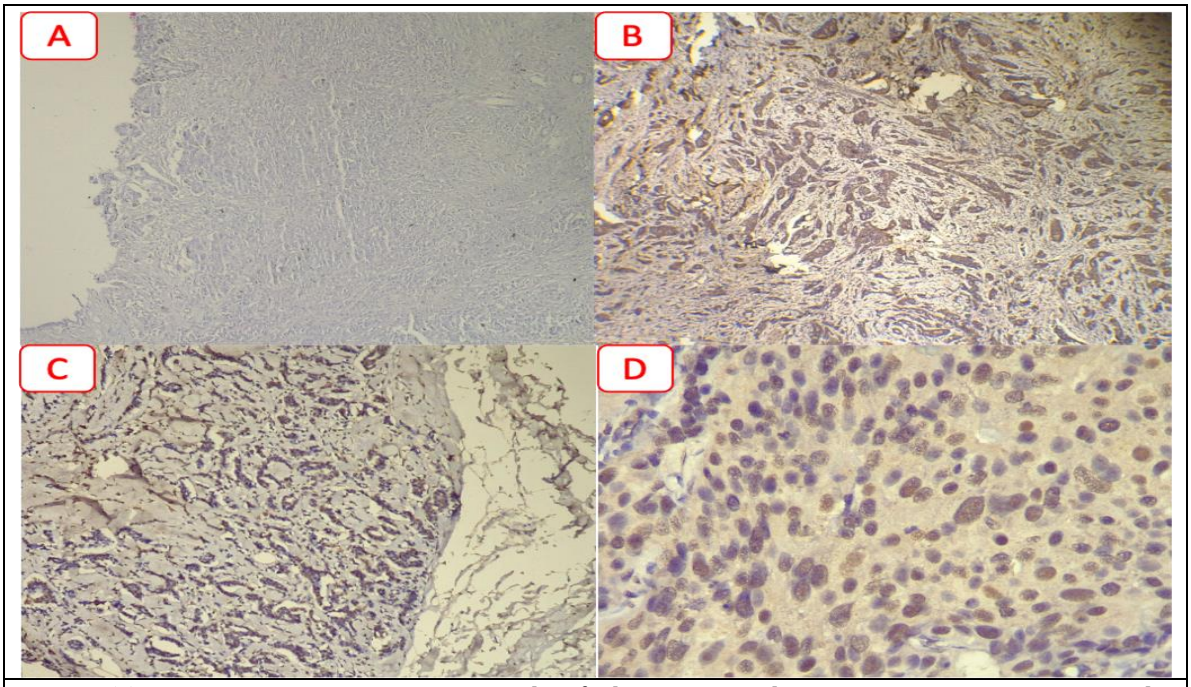

Figure (1): EBV EBNA-1 protein expression identified in IDC, cytoplasmic staining, A-Negative control, IHC, high power (x40), B-Moderate intensity, IHC, high power (x40), C-strong intensity, IHC, high power (x40), D-Weak intensity, IHC, high power (x40).

Discussion

The most prevalent histological type in this study was invasive ductal carcinoma (IDC), with 42/45 (93.3%) instances, followed by invasive lobular carcinoma (ILC) with 3/45 (6.7%). However, the histological grade showed that grade-2 differentiated breast cancer was found in (55.5% of the cases investigated), grade-3 in (25/45) (26.6%), and grade-1 in (8/45) (17.7%). It examined the relationship between histology kinds and grades and EBV. The most common kind was IDC, grade 1 in 7 (14%) patients, grade 2 in 25 (50%) individuals, and grade 3 in 14 (28%) patients. ILC was rare; 2 (4%) were grade 1, and 1 (2%) each grade 2 and 3. No

significant changes were found in breast cancer kinds or grades ($p > 0.05$). Current literature shows that IDC is the most frequent breast cancer subtype, mostly grade 2 and grade 3. However, invasive lobular carcinoma (ILC) is rare and usually has low-grade tumors (12,13). According to research, IDC accounts for 75%–80% of breast cancer instances and ILC for 10-15% (14,15). Typically, IDC is distributed by tumor grade: In 1991, Elston and Ellis reported 45% grade 2, 30% grade 3, and 25% grade 1. This distribution matches our analysis. However, 60–80% of ILC instances are grade 1 or 2 (16). Comparative studies show that ILC has a lower grade distribution than IDC. ILC is a low-grade "luminal A breast cancer prototype," according to (17). One ILC subtype had higher tumor grades than IDC, according to (18), while (19) revealed more aggressive HER2-positive ILC. Grade 2 IDC is more common than grade 1, while grade 3 is seen in younger individuals or more aggressive cases (20). ILC is usually G2, while LCIS is common (21). Due to its invasiveness and higher incidence rate, IDC is more likely to be identified as grade 3 than ILC. Many studies demonstrate that IDC is high-graded (22). The most common tumor grade in IDC is grade II, followed by grade III and grade I. Core biopsy grades correlate 69% with IDC surgical pathology (23). ILC is detected later and at a lesser degree, usually grade 1 or 2, making it less aggressive than IDC. Our findings are congruent with past breast cancer research, particularly with the distribution of histological grades in IDC and the less common ILC. Our investigation found a decreased incidence of ILC, which is consistent with the disease literature (24,25). The details of our ILC cases may reveal disparities in patient outcomes or treatment responses that the general knowledge literature does not cover in the context of the identified factors. The small number of individuals and possible interobserver grading variability limit this study. In the current study, breast cancer kinds and grades did not differ significantly, although a larger sample size may reveal more tendencies. Comparing our results to those of larger, population-based samples and examining the relationship between histological grade and other prognostic factors like tumor size, lymph node involvement, and hormone receptor status may yield more useful insights.

Conclusion: invasive ductal carcinoma and invasive lobular carcinoma are considered 64.3% of 70 blocks. More, the findings of the study indicate that the RNA representing EBNA-1 and EBV EBER was overexpressed in the population cohort that we studied.

Author Contributions

The study design and performed experiments were done by Thaer Saleh Sabor Al-Omary and Alaa Shamikh Hassan. In addition, all authors analyzed the data and wrote the manuscript.

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There are no funds for this study.

Ethics:

The study protocol was reviewed by the Human Ethics Committee of the College of Medicine, University of Misan, Iraq.

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Conflict of interest:

There is no conflict of interest.

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