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The potential role of Epstein-Barr Virus in breast cancer development

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ABSTRACT

We are looking into viral components that may contribute to breast cancer to find possible therapeutic targets. The Epstein-Barr virus (EBV), which has been found to cause nasopharyngeal carcinoma and Burkitt lymphoma, is thought to play a role in breast cancer. Our series' patients had a median age of 49, with nearly half being under the age of 49. T2 tumors (two to five centimeters in size) make up the vast majority of our collection (60%). Six percent of our patients showed lymph node involvement, with roughly the same number in the N1 and N2 stages (41.17% each). Only 17.64% of people are at the N3 stage. SBR II tumors were the most common (90%). Only 20% of patients have HER2 overexpression, whereas 73.33% have ER expression. EBV was found in 23.33% of breast carcinomas (7 cases/30) after oncoprotein LMP1 expression, but normal surrounding tissues tested negative. We discovered that overexpression of the HER2 protein is inversely related to the two HRs' expression. They have no relationship with EBV infection and, consequently, LMP1 expression. LMP1 expression was not shown to be linked with patient age, tumor grade, tumor size, or lymph node invasion.

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Introduction

Breast cancer is the most frequent disease in women globally, and it is the leading cause of mortality for women in developing nations (1). It is the second leading cause of mortality in developed countries, behind lung cancer (2). The illness is complicated and multidimensional, occurring when breast epithelial cells lose control of their development. Despite advances in therapy, breast cancer remains a major public health problem worldwide. It is vital to discover prognostic and predictive indicators, such as hormone receptors (HR) and the HER2 oncogene, to identify novel treatment targets. Steroid hormones, such as estrogen and progesterone, can have a major effect on breast tissue, resulting in hormone-sensitive or hormone-dependent tumors. Overexpression of HER2 is a frequent genetic change in this illness, and monoclonal antibodies targeting this receptor have emerged as an effective treatment strategy (3).

Age, histological subtype, tumor size, lymph node involvement, Scarff-Bloom-Richardson (SBR) histoprognostic grade, as well as the expression of hormone receptors or HER2 overexpression, serve as established indicators for breast cancer and offer vital insights into treatment strategies and prognostic assessments. Furthermore, viral infections are thought to be linked to several types of human cancer. Epstein-Barr virus (EBV), for example, is thought to have a role in the development of several cancers, including undifferentiated nasopharyngeal carcinoma and Burkitt lymphoma (4).

This research focuses on the Epstein-Barr virus's capacity to express a particular latent protein, LMP1, which is important in EBV's carcinogenic potential. Through its C-terminal domain, which is separated into three activating parts known as CTARs (C-terminal activating parts), LMP1 activates various signaling pathways, including NF-B, AP1, and STAT. CTAR1 and CTAR2 promote cell proliferation, prevent apoptosis, and promote cell immortalization via activating the NF-B pathway. CTAR3 stimulates VEGF (vascular endothelial growth factor) synthesis, which leads to invasion and metastasis. In Algerian patients, we are investigating a probable link between EBV infection and breast cancer.

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Materials and Methods

Biological material

The pathology staff at Tizi-Ouzou University Hospital provided us with tissue fragments of breast carcinomas collected from mastectomy specimens. These specimens served as biological materials for our study. It should be noted that the EBV serological profile was unknown at the time.

Patients considered

Thirty specimens were supplied by expert pathologists for the subsequent investigation. The goal of this study is to assess the level of expression of the EBV LMP1 protein and to determine whether the virus is present or not in these samples. In addition, we looked to see if HR and HER2 were expressed.

Immunohistochemistry

Tissues were fixed for 24 hours in buffered formalin (10% formaldehyde). Gradually rising alcohol concentrations (50, 70, 96, and 100%) dehydrated tissue (2 hours per bath). The alcohol is dissolved by xylene, allowing the clarity of the preparation (2 hours). The tissue was then immersed in liquid paraffin for two hours to recover the water that was lost during the dehydration procedure. The tissue within a solid block was then cut into thin slices of 3 to 5 microns. These were placed in a 37°C water bath before being spread across silanized slides. For about 12 hours, the blades are deparaffinized in an oven at 37 degrees Celsius. For tissue rehydration, the slides were immersed in xylene (2 x 5 minutes), then in alcohol at decreasing concentrations (100, 90, and 70%) for 3 minutes per bath, then in distilled water for 30 seconds. The formalin-masked epitopes were recovered by immersing the blades in an unmasking solution (citrate buffer at pH=6) and heated at 95-97°C for 30 to 40 minutes. After cooling, the slides were immersed in tubes containing distilled water and washing buffer for 5 minutes at a time. By immersing the slides in a solution containing hydrogen peroxide (H_2O_2). The endogenous background was reduced by blocking endogenous peroxidases for 5 minutes and then rinsed in distilled water and washed in a PBS (Phosphate-Buffered Saline) buffer for 5 minutes to remove H_2O_2. The tissue sample is treated for 30 minutes with 100 µl of the primary antibody (mouse anti-RE or anti-PR human monoclonal antibody) or (rabbit anti-human HER2 antibody) or (mouse anti-LMP1 antibody, clone CS. 1-4). The remaining slides were treated identically with a negative control reagent (negative control). The samples were washed for five minutes. 100 µl of secondary antibody is added for 30 minutes at room temperature. It is made up of dextran bound to horseradish peroxidase and secondary antibodies directed against mouse immunoglobulins in the case of HR and LMP1, or directed against rabbit immunoglobulins in the case of HER2). The slides were then cleaned with two washes.

On the slides, 100 µl of substrate buffer containing hydrogen peroxide was poured, followed by the addition of the chromogenic substrate of peroxidase, DAB (tetra hydrochloride of 3,3-diaminobenzidine). After a 10-minute incubation, the brown color indicated the antigen-antibody interaction.

Mayer's hematoxylin is used for contrast staining (1 to 5 minutes), which colors the nuclei purple-blue, the cytoplasm, and the supporting tissue (less intensely). The slides were rinsed with distilled water, and then immersed in an alcohol bath, and in xylene, to decolor the cytoplasm and connective tissue.

Ethical approval

The study was conducted following the Declaration of Helsinki, and approved by the Mouloud Mammeri University, Ethics and Deontology Commission of the Faculty of Biological Sciences and Agronomic Sciences (UMMTO/20/06/2021/Eth-Deon-A-041).

Statistic study

The statistical analysis for the tests involved the use of SPSS Statistics, version 25.0. (Fisher's exact test and Spearman's correlations) to identify any links between the factors under study.

Results

Descriptive study

Location and seat of the tumor

In our sample, 70% of the malignancies were detected in the left breast, reporting percentages of 53.13% and 60.4%, respectively, of tumors in the left breast. We did not observe any cases of bilateral involvement.

Patient age

The patient's average age is 48.33 years. The age group most heavily affected, accounting for 47% of cases, is 40 to 49 years old. Patients aged 20 to 29 and 70 to 79, on the other hand, had only one case (Figure 1).

Histological type

Infiltrating or Invasive Ductal Carcinoma is the most frequently occurring histological form, accounting for 77% of cases. Our analysis reveals that these uncommon types of breast cancer, which have a higher survival rate, represent 13.32% of cases. On the other hand, the proportion of mucinous carcinoma accounts for 3.33%.

TNM classification

The tumor size of T2 (2 cm < tumor ≤ 5 cm) is the most common in our data, accounting for 60% of the population. In our series, 57% of patients are at stage N+ and 43% are at stage N0. Among the 57% of patients with lymph node metastases (N+), 41% are at stage N1 (07 cases), 41% at stage N2 (07 cases), and only 17% at stage N3 (03 cases).

The majority of individuals (93.33% or 28) were assigned the Mx stage because of a lack of information re-

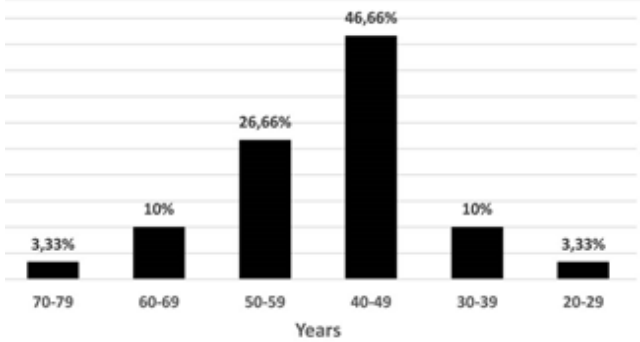

Figure 1. Proportions of breast cancer by age groups.

garding metastases. Only two patients (6.66%) did not have distant M0 metastases, and no patient was categorized as stage M. This lack of information results in a significant disparity with the literature.

The most prevalent SBR grade discovered is grade II (90% of cases), followed by grade I (6.66% of cases) and grade III (3.33% of cases). This apparent prevalence of grade II.

Expression of Hormone Receptors (HR)

The nuclear staining indicated the presence of this Hormone receptor (HR), with a positivity threshold of 10%. Nuclei that are stained brown are considered positive for HR, while those that are blue are negative (Figure 2). Hormone therapy is frequently utilized in the treatment of hormone receptor-positive breast cancer.

Expression of the Estrogen receptor (ER) and Progesterone receptor (PR)

The ER is expressed by 73.33% of patients. As a result, only 26.66% (8 out of 30 instances) are RE negative (Figure 3).

Expression of the HER2 oncogene

The tumor cells showed a strong, complete brown, mesh-like membrane staining in response to HER2 immunohistochemistry, as illustrated in Figure 4.

Of our patients, 20% expressed the HER2 protein (score 3), while 79.99% had a negative HER2 profile (53.33% score 0 and 26.66% score 1), and no uncertain cases were found (score 2), as shown in Figure 5.

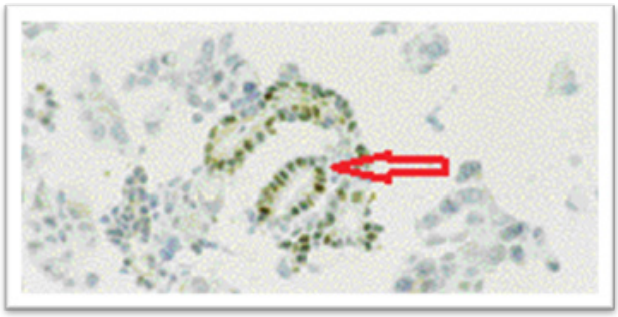

Figure 2. Micrograph of a cluster of ER-tumor cells with positive internal control (arrow) at x40 magnification.

A. Distribution of cases according to the expression of ER

B. Distribution of cases according to PR expression

Figure 3. Distribution of cases according to the ER (A) or PR (B) expression (in yellow).

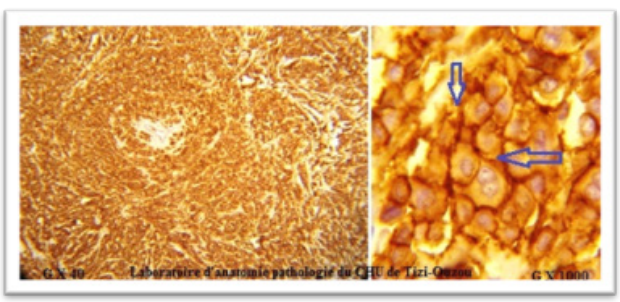

Figure 4. Photomicrograph of a cluster of HER2 positive tumor cells (score 3+) showing strong and complete staining (arrow) at low (left) and high (right) magnification.

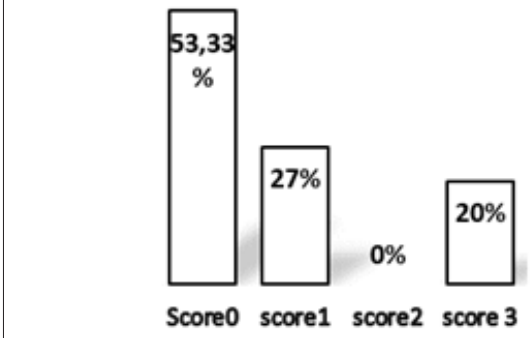

Figure 4. Distribution of cases according to HER2 status.

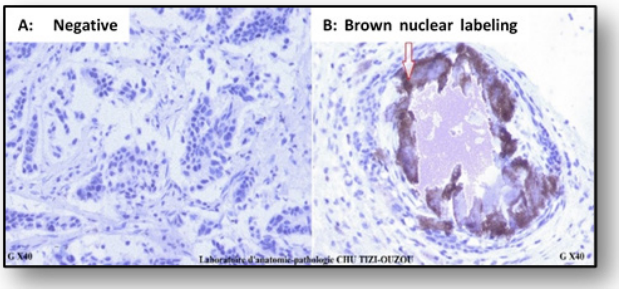

Figure 6. Microphotograph of a cluster of tumor cells (A negative control, B brown nuclear labeling with LMP1).

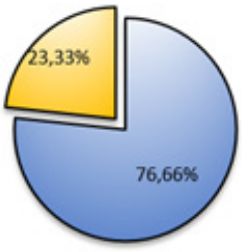

Figure 7. Distribution of cases according to the LMP1 profile.

Expression of the EBV LMP1 protein

The expression of EBV LMP1 protein was evaluated using nuclear staining with LMP1 antibody. The term "LMP1-positive expression" was used to describe this staining pattern regardless of the intensity of staining or the percentage of cells that were stained (Figure 6).

The presence of the EBV virus in mammary carcinomas was confirmed by the expression of the LMP1 protein, which was found in 23.33% of tumor samples but not in the adjacent healthy tissue (Figure 7).

There was no control tissue identified, and all patients with EBV (6.7%) had advanced tumor grades (II and III).

Analytical study

Relationships and correlations between the novel prognostic factors and the expression of the oncoprotein LMP1

In summary, we found a high link and positive correla-

tion between the expressions of the two HRs, which are negatively associated with HER2 protein overexpression. We were unable to determine a statistically significant relationship between the expression of the oncoprotein LMP1 and the three prognostic variables, namely HR and HER2. However, as indicated by the summary of our results in Table 1, not all EBV-positive patients overexpressed HRE2, despite being mostly HR-positive.

A statistically significant correlation was discovered between the expression of ER and PR, indicated by a Cramer's V score of 0.75. Consequently, a large proportion of patients who are ER+ also exhibit PR+ expression. As shown in Figure 8, 66.66% of patients have both an ER and a PR profile (ER+/PR+). Several (23.33%) patients had an ER-/PR- profile, two cases had an ER+/PR- profile, and one had an ER-/PR+ profile. Our study confirmed this relationship with a significant P value and a Cramer's V score greater than 0.70, indicating a strong correlation between the expression of these two receptors. Additionally, we observed a robust inverse association between ER expression and HER2 overexpression, with a correlation value of -0.75.

One pattern is also evident in the histogram shown in Figure 9. Specifically, 60% of the patients who express ER do not exhibit HER2 overexpression (i.e., have a RE+/HER2- profile), while 56.6% of the patients who express PR are also HER2-negative. Furthermore, the RE+/HER2+ profile was observed in 13.33% of cases.

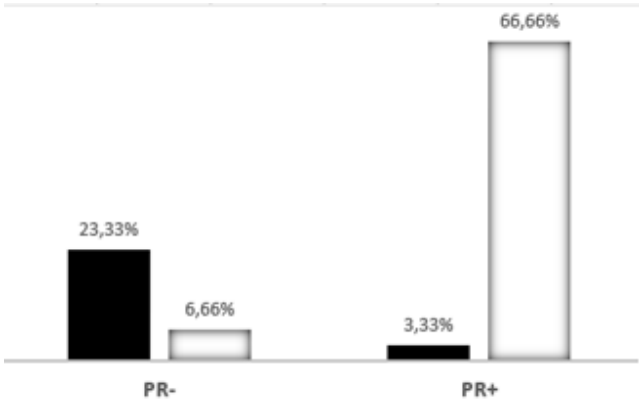

Figure 8. Correlation between HR: Clear color (ER+), Dark color (ER-).

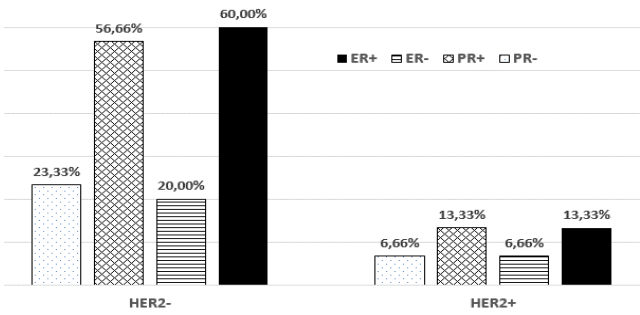

Figure 9. HR and HER2 expression profiles.

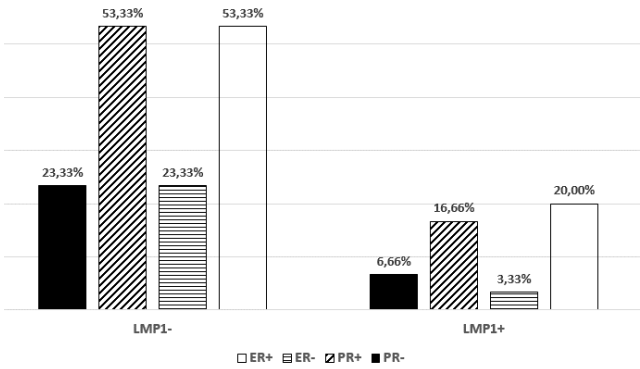

Figure 10. HR and oncoprotein LMP1 expression profiles.

Regarding PR/HER2, we did not find a statistically significant relationship. However, there seems to be an inverse correlation with a non-significant SPEARMAN coefficient of -0.36. This inverse correlation aligns with the positive ER/PR correlation and the negative ER/HER2 correlation. Based on these findings, it can be inferred that the PR/HER2 correlation is likely negative.

In terms of the relationship between HR expression and the presence of EBV, no statistical correlation was observed. Specifically, for ER, the P value was 0.638 (>0.05) with a Cramer's V of 0.154, and for PR, the P value was 1 with a Cramer's V of 0.017. Their findings indicate that the presence of EBV is not associated with the upregulation of steroid receptors. In contrast, our study found that

Table 1. Anatomopathological characteristics of EBV-positive tumors.

Case	Age	Histological case	TNM	SBR	ER	PR	HER2	LMP1
1	36	CM	T1cN0Mx	II	+	+	-	+
2	45	CCI	T1cN0Mx	II	+	+	-	+
3	53	CCIn Situ	T2N0Mx	II	-	-	-	+
4	58	CCI	T3N2aM0	II	+	+	-	+
5	58	CCI	T2N1aMx	II	+	+	-	+
6	44	CCI	T1cN0Mx	II	+	+	-	+
7	42	CCI	T2N0Mx	I	+	+	-	+

Table 2. Relation between LMP1 expression and the breast carcinoma risk factors.

	P value	Correlation Coefficient	Signification
LMP1-Age	0,680	-0,078	Not significatif
LMP1-SBR	0,564	0,19	Not significatif
LMP1-T	0,442	-0,146	Not significatif
LMP1-N	0,092	-0,313	Not significatif

most patients expressing HR do not have LMP1 (Figure 10), where 53.33% of patients had an HR+/LMP1- profile.

There was no statistically significant relationship found between the expression of the viral protein LMP1 and overexpression of the HER2 protein, as indicated by a *P* value = 0.290> 0.05 and a Cramer's V score of 0.276. Similarly, no significant correlation was observed. No correlation was found between the presence of EBV and HER2 overexpression, especially since none of the patients in our series exhibited an LMP1+/HER2+ profile.

Correlation between the expression of LMP1 and the classic prognostic factors.

We did not observe any statistically significant correlation in our series between the expression of LMP1 protein and the conventional prognostic factors, such as patient age, SBR grade, tumor size, or lymph node invasion (Table 2).

Discussion

Location of the tumor

Almost all samples from malignancies (70%) were detected in the left breast, which is in line with the results of previous studies (3,4), reporting percentages of 53.13% and 60.4% of tumors in the left breast respectively. This phenomenon may be explained by the breastfeeding practices hypothesis (7). Studies have shown that women who breastfeed their children for at least 12 months may increase their life expectancy by 26% (8). Thus, if the right breast is used for breastfeeding more frequently, the left breast may become more vulnerable to cancer. However, Mansouri (8) observed a different tumor pattern, the right breast was more affected. On the other hand, we did not observe any cases of bilateral involvement, which is consistent with the literature reporting that only 1 to 2% of individuals develop cancer on both sides (8,9).

Patient age and samples

The mean age of the patients is around 49 years, which is similar to the average age reported in Morocco (ranging from 48.1 to 50.7 years) (9,10,11). However, this mean age is lower than that reported in a French study, which was approximately 52 years (12). The most affected age group, accounting for 47% of cases, is between 40 and 49 years old. This is consistent with Diallo's study conducted in Mali in 2007 (13). On the other hand, the age groups with the lowest number of cases include those aged 20 to 29 and 70 to 79, with only one case each. This finding might be explained by the lower rate of older women consulting and lower life expectancy in Algeria.

Infiltrating or Invasive Ductal Carcinoma is the most frequently occurring histological form (77%). In France, this proportion of this kind of tissue is slightly higher (82%) (14); an Iranian study (15) reported a proportion of 96.3%.

Invasive lobular carcinoma accounted for 10% of our cases. This agrees with the Cordina-Duverger study (14). Different other studies have reported a proportion of this tumor up to 15% of all cases, distinguished by its HR positivity and HER2 negativity (16). Our analysis revealed that these uncommon types of breast cancer, have a higher survival rate. Our data were higher than those of Cordina-Duverger (16) (4%). On the other hand, the proportion of mucinous carcinoma, which accounts for 3.33%, is similar to the literature estimate of 4% for all invasive breast tumors (17).

The tumor size of T2 (2 cm < tumor ≤ 5 cm) is the most common in our data. Marty et al. (18), in France, reported a ratio of 45.5%, which is close to but lower than our findings. Moroccan research has a success rate of 50% (19). Tx, T0, and T4 stages were not present in our dataset, and the T3 stage was only 13.33%, lower than in previous studies (5,13,20) with 60.9%, 75.8%, and 49.4 % respectively. The absence of stage T4 and the low number of T3 patients would be due to preventive actions in our region.

In our patient cohort, 57% of individuals were found to be in stage N+ while 43% were classified as stage N0. This distribution deviates from the prevailing trend observed in most studies, where approximately 20% of cases are categorized as stage N0 and 80% as stage N+. This was consistent with the findings of Keita (10) and Diallo (13). Patients' awareness and screening consultations may explain this trend, but during the COVID-19 pandemic, there may have been a step back, as demonstrated by an American study, which reported a 32-day average delay in treatment initiation for breast cancer, followed by increased mortality rates at 5 and 10 years, depending on disease stage (21).

The limitation of our study is that the majority of the patients were assigned the Mx stage because of a lack of information regarding metastases. Only two patients (6.66%) did not have distant M0 metastases, and no patient was categorized as stage M. This lack of information results in a significant disparity with the literature. Two Moroccan studies (22,23), which did not include any patients in stage Mx, both agree on a majority proportion of set M0, with 87.3% and 95%, respectively, compared to 12.7% and 5%.3.1.5. SBR grade

Grade II is the most common SBR grade, accounting for 90% of cases. Grade I is less common, at 6.66%, and grade III is the least common, at 3.33%. This prevalence of grade II is consistent with other studies in the literature (22,23). Our study on breast cancer found that grade II is the most common SBR grade, while other studies have reported a higher prevalence of either grade I or grade III. This discrepancy may be because our study included samples from tumors in all stages of development, while the other studies only included samples from early-stage tumors.

Expression of Hormone Receptors (HR)

The identification of hormone receptors (HR) is a critical aspect of selecting appropriate treatment (prognostic factor). The presence or absence of these receptors is used to anticipate the response to hormone therapy (predictive factor) (24). The nuclear staining indicated the presence of this receptor, with a positivity threshold of 10%. The use of nuclear staining to identify hormone receptor-positive cells is a well-established diagnostic tool that has revolutionized cancer treatment. The significance of detecting hormone receptors in determining the most effective treatment for a patient.

The ER was expressed in 73.33% of patients. Other studies reported averages from 60% to 70%. However, our findings were higher than those found in other Arab countries such as Tunisia (25), which found a percentage of positivity of no more than 59.4%, and Jordan (26), which

found an even lower rate of positivity of 53%. Furthermore, Anderson et al. (27) and Chow et al. (28) discovered lower expression levels than we did. However, our findings are consistent with French investigations (29,30), with an ER expression not exceeding 70%.

Our findings concerning PR were quite similar to those of Hammas's Moroccan study (22), which recorded 71.4% positive cases.

Expression of the HER2 oncogene

20% of our patients expressed the HER2 protein, while 79.99% had a negative HER2 profile. Of the patients with a negative HER2 profile, 53.33% had a score of 0, and 26.66% had a score of 1. No patients had an uncertain HER2 profile (score 2). These results are consistent with previous studies reporting a positive rate of 20-30% (31,32).

We observed 20% of cases positive in HER2. This was slightly lower than that reported in several other studies, including an Australian study (33), which reported a positivity rate of 26%, as well as a Tunisian study (25), and a Jordanian one (26). However, a Moroccan study (9) found a significantly higher positivity rate of 87%.

A positive correlation has also been observed in several other studies, such as those conducted by Almasri et al. (28) in Jordan and Ayadi et al. (25,26) in Tunisia. Consequently, a large proportion of patients who are ER+ also exhibit PR+ expression. In the present study, 66.66% of the patients have both an ER and a PR profile (ER+/PR+), which is a higher proportion than reported in previous studies (22,26) which reported a lower rate of less than 39.5%. 23.33% of patients had an ER-/PR- profile, two cases had an ER+/PR- profile, and one had an ER-/PR+ profile. This is because the expression of PR is heavily reliant on ER expression, which explains why tumors that are ER-negative tend to lack PR expression and vice versa. Our study confirmed this relationship with a significant P value and a Cramer's V score greater than 0.70, indicating a strong correlation between the expression of these two receptors. Additionally, we observed a robust inverse association between ER expression and HER2 overexpression, with a correlation value of -0.75. Specifically, 60% of the patients who express ER do not exhibit HER2 overexpression (i.e., have a RE+/HER2- profile), while 56.6% of the patients who express PR are also HER2-negative. These findings suggest that HR+ patients are more likely to be HER2-negative.

Our findings are consistent with previous research that has found a higher prevalence of HER2 score 3 cases in patients who are RE- compared to those who are RE+. This trend has been observed in several studies (32,34,35). According to Eliss et al. (36), HER2 amplification is detected in around 35% of RE- cases, while it is only present in 10 to 15% of RE+/HER2+ cases. In our study, the RE+/HER2+ profile was observed in 13.33% of cases. This phenomenon can be attributed to the suppressive effect of estrogen on the transcription of HER2, as HER2 can induce cell proliferation in the absence of the RE pathway.

Expression of the EBV LMP1 protein

The presence of the Epstein-Barr virus (EBV) in mammary carcinomas was confirmed by the expression of the LMP1 protein in 23.33% of tumors. This finding is consistent with other studies, such as a study conducted in Jordan in 2013 (37) that found a 26% positivity rate using two detection methods, PCR and immunohistochemistry. Other studies (38,39) found a positivity rate of 27.02% in tumor tissues compared to only 11.42% in controls. Our immunohistochemistry analysis of breast cancer samples revealed the presence of EBNA1 protein expression with granular nuclear staining in 26% of cases.

Mofrad et al. (40) found the lowest percentage of EBNA positive at 6.7% of breast cancer cases associated with EBV. Furthermore, all the patients had advanced tumor grades (II and III). While a Lebanese study (41) reveals an EBV presence rate of roughly 40%.

Even though multiple studies have shown EBV detection in breast cancers with different proportions (27.77% (42), 29.33% (43), 35% (44), and 46% (45)), Other studies (46,47,48,49), could not demonstrate the presence of the virus in these tumors. The difference in the results described in the literature could be attributed to the variety of techniques employed to detect the virus (IHC, PCR, ISH), the varied target EBV proteins examined, or even the genetic/ethnic origin of the population studied. It appeared that the age at which the first EBV infection occurred may potentially have an impact (50).

Because it is not found in all breast cancer cells, we can conclude that EBV plays a role in breast oncogenesis but not as a key etiological factor. Indeed, even in situations of nasopharyngeal carcinomas when the role of EBV is firmly established, not all tumor cells are responsive to the antibody (51). In the case of breast cancer, it would rather act as an agent capable of modifying the behavior of cells that have already been transformed, giving them a more aggressive phenotype (52). Sharaf and Gomaa (53), discovered that EBV+ breast cancers are more aggressive than other breast cancers. Other authors have recently revealed that LMP1 gene expression is related to an invasive phenotype (1).

In terms of the relationship between HR expression and the presence of EBV, no statistical correlation was observed. These results are consistent with other studies (52,54). These findings indicate that the presence of EBV is not associated with the upregulation of steroid receptors. However, this conflicts with studies that have shown a strong negative correlation, such as those previously conducted (53). Hachana et al. (54), and Mazouni et al (55). This suggests that EBV is more frequently detected in HR-negative breast tumors, which are generally more aggressive (56). In contrast, our study found that most patients expressing HR do not have LMP1, whereas 53.33% of patients had an HR+/LMP1- profile. However, we were unable to conclude the opposite idea that patients expressing LMP1 do not have HR because the majority of patients in our sample were HR+. A larger survey with more participants may reveal the inverse correlation noted in several studies.

There was no statistically significant relationship found between the expression of the viral protein LMP1 and overexpression of the HER2 protein. Similarly, no significant correlation was observed. Our findings are consistent with other results (52,57). However, the literature suggests that EBV infection of certain breast cancer cell lines can activate HER2/HER3 signaling pathways. It is, therefore, surprising that no correlation was found between the presence of EBV and HER2 overexpression, especially since none of the patients in our series exhibited an LMP1+/

HER2+ profile.

Our study did not find any statistically significant correlation between the expression of the LMP1 protein and the conventional prognostic factors for breast cancer, such as patient age, SBR grade, tumor size, or lymph node invasion. This finding is consistent with other studies, such as those by Fina et al., (57), Preciado et al. (58), Hachana et al. (54), and Mohammedizadeh et al.(59), which also found no statistically significant correlation between EBV infection and these prognostic factors. Chu et al. (60) and Khabaz (37) also found no significant association between the development of EBV breast cancer and these factors.

In Egypt, Sharaf and Gomaa conducted a study (54) that did not find a significant correlation between the presence of EBV and age. However, the study did reveal significant associations between the expression of the EBV genome and unfavorable prognostic factors, such as high tumor grade and lymph node involvement. These findings align with a previous study (61) that reached a similar conclusion. Another study by Fawzy et al. (62) established a connection between the presence of EBV in tumors and lymph node invasion, suggesting that EBV might contribute to the development and altered behavior of aggressive carcinomas with a heightened potential for metastasis. These collective findings imply an association between EBV and the increased metastatic potential of tumors.

Aggressive characteristics were observed in breast cancers expressing EBV (56), particularly those with high tumor grade. Similarly, Murray et al. (52) found that EBV-positive tumors were more likely to exhibit high grades, involve multiple lymph nodes, and have larger sizes. The study by Sharaf and Gomaa (54) also noted a similar pattern in tumor size, where EBV was absent in small T1 tumors (<2 cm), and all EBV-positive carcinomas were larger T2 or T3 tumors (>2 cm), although this difference did not reach statistical significance. Additionally, a study by Glenn et al. (63) discovered a correlation between the presence of EBV in breast tumors and a younger age at diagnosis. While the existing literature on the association between EBV and poor prognostic factors is somewhat inconsistent, these findings collectively suggest an overall unfavorable prognosis for the disease.

Conclusion

The association between Epstein-Barr virus (EBV) and breast cancer is a subject of ongoing research, and the role of EBV in breast cancer development is still not fully understood. While there have been some studies suggesting a potential link, the evidence for active EBV infection in breast cancer tissues, specifically through the expression of EBV latent membrane protein 1 (LMP1), is limited. Currently, there is no consensus in the scientific community regarding the presence of active EBV infection in breast cancer. Nevertheless, our analysis of a small sample of patients (23.33%) has revealed a correlation between invasive breast cancer and the presence of EBV. The virus was only found in tumor cells, suggesting its potential involvement in the development of certain forms of breast cancer. However, EBV does not seem to have a significant impact on the major clinicopathologic correlation and prognostic factors of breast cancer. Given these results, we propose that immunotherapy or antiviral therapies that target EBV may be beneficial in treating some types of invasive breast cancer.

Author Contributions

Conceptualization, K.G., L.T., and A.M.; methodology, K.H., A.K., and M.M.; validation, L.T., S.O., and Y.B.; formal analysis, K.G., and A.M.; investigation, A.K., and K.H.; resources, M.S.B., M.M., and L.T. data curation, S.O., M.S.B., and Y.B.; writing—original draft preparation, M.S.B., and M.M.; writing—review and editing, M.S.B., K.H. and A.E.; supervision, Y.B. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The study was conducted following the Declaration of Helsinki, and approved by the Mouloud Mammeri University, Ethics and Deontology Commission of the Faculty of Biological Sciences and Agronomic Sciences (UMMTO/20/06/2021/Eth-Deon-A-041).

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

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Conflicts of Interest

The authors declare no conflict of interest.

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