Are Mouse Mammary Tumor Virus and Bovine Leukemia Virus Linked to Breast Cancer among Jordanian Women?

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Abstract: The investigation into the potential association between retroviruses and breast cancer (BC) presents a fascinating area of research. In this study, the focus was on assessing the presence of two retroviruses, Mouse Mammary Tumor Virus (MMTV) and Bovine Leukemia Virus (BLV), in BC samples and exploring their relationship with relevant clinicopathological variables. The study involved analyzing BC samples from 103 Jordanian female patients diagnosed with BC, as well as breast tissue samples from 25 control patients without evidence of breast malignancy. Real-time PCR was used to investigate the evidence of MMTV and BLV infection in these samples, and the findings were then correlated with various clinicopathological characteristics of BC. The results showed that BLV was detected in 19 (18.4%) of the BC samples, while MMTV was detected in only seven (6.8%). Importantly, none of the control samples tested positive for MMTV or BLV. Additionally, MMTV/BLV co-infections were reported in 1.9% of the BC cases. However, the analysis did not reveal any statistically significant associations between the presence of these retroviruses and various clinicopathological variables, such as age, molecular subtypes of BC, stage, grade, lymph node involvement, tumor size, smoking status, or family history. Despite these findings, it is crucial to acknowledge that further investigation with a larger cohort is necessary to establish a clearer association and elucidate the underlying mechanisms that may explain the exact role of retroviruses in breast carcinogenesis. This study provides insights into the potential infection by MMTV and BLV of BC and lays the groundwork for future research in this area.

Keywords: MMTV; BLV; breast cancer; nested PCR; Jordan

1. Introduction

Breast cancer (BC) represents a substantial global health challenge, with consistent prevalence rates across different regions worldwide, constituting around 25% of all cancers and contributing to approximately 15% of cancer-related fatalities among women [1]. In Jordan, BC poses a significant healthcare issue, emerging as the primary malignancy among females and resulting in the highest cancer-related female mortality rates in the country [2].
BC exhibits significant heterogeneity, comprising various subtypes characterized by distinct genetic backgrounds, pathogenic mechanisms, response to treatment, and prognostic outcomes. This heterogeneity suggests the involvement of other causative factors in the development of BC. Genetic mutations play a crucial role in the multistep process of BC oncogenesis, including both sporadic and familial cases [3]. However, familial predisposition accounts for only a small fraction of BC cases, highlighting the importance of considering other acquired or environmental factors.

In 2022, it was reported that there were 164,000 women living with metastatic BC in the United States, indicating a concerning increase in metastatic BC cases [4]. Besides genetic factors, environmental influences such as alcohol consumption, smoking, lifestyle choices, obesity, and body mass index also contribute to BC incidence and prognosis [5,6]. Approximately 16% of human cancers are attributed to infections, including oncogenic viruses like human papillomavirus (HPV), which is implicated in cervical and oropharyngeal cancers [9,10], viruses such as human immunodeficiency virus (HIV), which weaken the host immune responses [11], and hepatitis C virus (HCV) and hepatitis B virus (HBV), which induce chronic inflammation and tissue damage [12].

Initially, a viral etiology of BC was not widely accepted until the discovery of the Mouse Mammary Tumor Virus (MMTV), a retrovirus transmitted from mothers to offspring during lactation [13]. Subsequent evidence revealed the presence of the MMTV gene sequence in up to 40% of infiltrating BC cases [14]. Several studies indicated a strong association between the presence of MMTV and the progression of BC [14–17]. Notably, MMTV was also detected in the saliva and salivary glands of BC patients [18]. Interestingly, MMTV was found to be nearly absent in cases of hereditary BC compared with sporadic cases, suggesting potential implications for BC exacerbation [19].

Bovine Leukemia Virus (BLV) presents a potential risk to both animals and human health [20]. However, the question of its zoonotic potential remains ambiguous, requiring further investigation to elucidate its relationship with humans. Numerous studies have aimed to establish a connection between BLV and BC [21,22]. Although genome fragments of BLV have been identified in BC patients, the exact etiology has yet to be definitively determined [23,24]. In vitro studies have demonstrated the capability of BLV to infect human mammary cells, and BLV antibodies have been detected in human blood, underscoring the potential threat posed by BLV transmission and propagation in humans [25].

BLV DNA was detected in human BC, and a meta-analysis of case-control studies investigating the association of BLV with healthy and cancerous breast tissue was conducted [21,24]. Additionally, BLV was found to be present in a significant proportion of cancerous and precancerous breast tissue samples, emphasizing the importance of exploring BLV zoonosis [26]. However, conflicting results have emerged from various countries, with some studies failing to find a link between BC and BLV [27,28].

The aim of this study was to investigate the association of MMTV and BLV infections with BC cases among Jordanian women.

2. Materials and Methods
2.1. Sample Collection
A retrospective review of medical records from King Abdullah University Hospital (KAUH) was conducted to screen patients diagnosed with BC between 2018 and 2022. The inclusion criteria consisted of patients aged 20 to 80 years, histologically confirmed with BC, who had not undergone radiotherapy or chemotherapy before tumor excision, and whose samples tested positive for GAPDH. A total of 103 formalin-fixed paraffin-embedded (FFPE) tissue blocks from BC patients and 25 samples from nonmalignant breast lesions as controls were collected from KAUH in Irbid, Jordan. All research activities con-
duced within this study adhered to ethical guidelines approved by the Hashemite University Institutional Review Board (IRB) (Approval No. 7/14/2020/2021) and KAUH IRB (No. 42/151/2022).

2.2. Sample Processing and RNA Extraction

Each tissue block was sliced into 20 μm sections using a microtome, and each section was placed in a 1.5 mL microcentrifuge tube. Initially, the samples underwent deparaffinization by adding 1 mL of xylene to remove paraffin residues from all FFPE tissues. The tube was vortexed for ten seconds and then centrifuged for 2 min at maximum speed to separate the supernatant, which was carefully removed using pipetting. Following deparaffinization, 1 mL of 100% ethanol was added to the pellet, followed by vortexing and centrifugation for 2 min at maximum speed. The supernatant was then discarded, and 1 mL of 90% ethanol was added to the pellet, followed by vortexing and centrifugation for 2 min at maximum speed. The supernatant was also discarded, and the samples were then air-dried for 10 min.

RNA extraction was conducted using the RNeasy® FFPE Kit (Cat. No. 73504, QIAGEN, Fenlo, The Netherlands) according to the manufacturer’s instructions. Briefly, following incubation, 150 μL of Buffer PKD and 10 μL of Proteinase K were added and mixed by pipetting, and the mixture was then incubated at 56 °C for 15 min, followed by incubation at 80 °C for the same duration. Following this step, the samples were immediately placed on ice and incubated for 3 min, followed by centrifugation at 13,500 rpm for 15 min. The supernatant was carefully transferred to a new microcentrifuge tube to which 25 μL of DNase Booster Buffer and 10 μL of DNase I stock solution were added. The tube was inverted to ensure thorough mixing, briefly centrifuged, and then incubated at room temperature for 15 min.

Afterward, 500 μL of Buffer RBC was added to the sample and thoroughly mixed. Then, 1200 μL of 100% ethanol was added and mixed well. Subsequently, 700 μL of the sample was transferred to the RNeasy MinElute spin column and centrifuged for 1 min. This step was repeated until the entire sample was processed and all RNA was bound to the filter of the spin column. Next, the MinElute spin column was washed twice with 500 μL of Buffer RPE. RNA was eluted with 25 μL of RNase-free water and stored at −80 °C for future experiments. RNA concentration was measured using Qubit™ RNA HS Buffer with Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions.

2.3. Reverse Transcription and Real-Time PCR for MMTV detection

The RNA was converted to cDNA using the QuantiTect Reverse Transcription Kit (Cat. No. 205311, QIAGEN, Fenlo, The Netherlands) according to the manufacturer’s instructions. Briefly, The RNA template was initially cooled on ice, and then a mixture of gDNA Wipeout Buffer and RNase-free water was added. After that, the template RNA was added, and the reaction tubes were incubated briefly at 42 °C. For multiple samples, a master mix containing Quantiscript Reverse Transcriptase, RT Primer Mix, and Quantiscript RT Buffer was prepared and added to each tube. The total reaction volume was 20 μL. The tubes were centrifuged, followed by incubation at 42 °C for 15 min and then at 95 °C for 3 min to complete the reverse transcription process. cDNA was stored at −20 °C for future experiments. DNA concentration was measured using Qubit™ DsDNA HS Buffer with Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions.

Initially, a conventional polymerase chain reaction (PCR) was conducted to determine the optimal annealing temperature for each primer pair. Subsequently, the QuantiTect SYBR® Green PCR Kit (Cat. No. 204143, QIAGEN) was used to amplify the target DNA for each sample, employing the following forward primer ENVF (5′-ACCATCCTGCYTCAT-3′) and reverse primer ENVR (5′-CCCATCCTGCYTCAT-ACCAT-3′) (Figure S1). Each run contained standardized positive and negative controls.
PCR products were electrophoresed on a 2% agarose gel stained with ethidium bromide and visualized using a UV gel documentation system (Alpha Innotech, San Leandro, CA, USA).

2.4. Reverse Transcription and Nested PCR for BLV Detection

The RNA was converted to cDNA, as described in the previous section. To initially assess the quality of the extracted DNA samples, all samples underwent PCR using GAPDH primers. The GAPDH primers used were as follows: forward primer GAPDH-F: (5′-GAGTCAACCGATTGTTGCTG-3′) and reverse primer GAPDH-R: (5′-TTGATTTTGAGCGGATCTCG-3′), generating a PCR product of 237 bp. Additionally, for the detection of BLV, 50 ng of DNA was combined with a standard PCR mix supplemented with the tax gene outer forward (5′-CTTCGGGATCCATTACCTGA-3′) and reverse (5′-GCTCGAAGGGGAAGTGAAG-3′) primers. The cycling conditions were initial denaturation at 95 °C for 10 min, then 40 cycles at 95 °C for 15 s, 50 °C for 60 s, and 72 °C for 60 s. Subsequently, for the nested PCR, 5 μL of the previous PCR product was transferred to a new microtube containing the tax inner forward (5′-ATGTCCGATCGCTGGG-3′) and reverse (5′-CATCGCGGTCGCTGG-3′) primers. The cycling conditions were the same, except for the annealing temperature, which was adjusted to 56 °C. Each run contained standardized positive and negative controls. PCR products were electrophoresed on a 2% agarose gel stained with ethidium bromide and visualized using a UV gel documentation system (Alpha Innotech, San Leandro, CA, USA) and as shown in Figure S2.

2.5. Statistical Analysis

The statistical analysis was carried out using SPSS version 22. The Chi-square test was employed to assess variations in proportions, with statistical significance determined at a chosen level of $p < 0.05$.

3. Results

3.1. Clinicopathological Characteristics among the Study Cohort

In our final analysis, the breast sample cohort comprised 103 tumor samples, with a median age of 56 years serving as the criterion for patient stratification into two groups. Molecular subtyping revealed that 65.0% (67/103) of the samples were luminal A (ER+ and/or PR+ with HER2-), 19.4% (20/103) were luminal B (ER+ and/or PR+ with HER2+), 5.8% (6/103) were HER2+ positive only (ER and PR both negative), and 9.7% (10/103) were classified as triple-negative breast cancer (TNBC) lacking expression of hormonal receptors. Furthermore, clinical staging indicated that 44.7% (46/103) were stage I, 17.5% (18/103) were stage II, 14.6% (15/103) were stage III, and 23.3% (24/103) were categorized as stage IV. Lymphovascular invasion was detected in over half of the samples (53.4%; 55/103), while lymph node involvement with cancerous cells was observed in 70.9% (73/103) of cases.

3.2. Detection of BLV and MMTV using PCR

Our data showed that 19 out of 103 samples were found to be positive for BLV, constituting 18.4% of the total sample size (Table 1 and Figure S2). Conversely, only seven samples (6.8%) tested positive for MMTV (Table 2, Table S1 and Figure S1). Notably, our data revealed that co-infection of BLV and MMTV occurred in two samples (1.9%). Moreover, none of the normal breast samples demonstrated positivity for BLV or MMTV. These differences observed among normal breast samples were statistically nonsignificant ($p > 0.05$).
Table 1. Clinicopathological association of BLV status among malignant breast samples (n = 103). BLV: Bovine Leukemia Virus; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; LV: lymphovascular invasion; LN: lymph node involvement; +: positive; -: negative.

<table>
<thead>
<tr>
<th>BLV</th>
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<th>Negative</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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<td>12</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>&gt;56</td>
<td>7</td>
<td>43</td>
</tr>
<tr>
<td>Luminal A</td>
<td>ER+/PR+</td>
<td>11</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>HER2-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal B</td>
<td>ER+/PR+</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>HER+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER+</td>
<td>ER-/PR- &amp; HER+</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>TNBC</td>
<td>ER-/PR-</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>HER2-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stage I</td>
<td>12</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Stage II</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Stage III</td>
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<td>13</td>
</tr>
<tr>
<td></td>
<td>Stage IV</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Grade 1</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Grade</td>
<td>Grade 2</td>
<td>12</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Grade 3</td>
<td>5</td>
<td>31</td>
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<tr>
<td>Lymphovascular Invasion</td>
<td>Present</td>
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<td>43</td>
</tr>
<tr>
<td></td>
<td>Not identified</td>
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<td>41</td>
</tr>
<tr>
<td>Lymph node involvement</td>
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<td>25</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
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<td>59</td>
</tr>
<tr>
<td>Tumor size</td>
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</tr>
<tr>
<td></td>
<td>&gt;3 cm</td>
<td>7</td>
<td>43</td>
</tr>
<tr>
<td>Smoking</td>
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<td>2</td>
<td>12</td>
</tr>
<tr>
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<td>No</td>
<td>17</td>
<td>72</td>
</tr>
<tr>
<td></td>
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<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Family history</td>
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<td>64</td>
</tr>
<tr>
<td></td>
<td>Undetermined</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2. Clinicopathological association of MMTV status among malignant breast samples (n = 103). MMTV: Mouse Mammary Tumor Virus; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; LV: lymphovascular invasion; LN: lymph node involvement; +: positive; -: negative.

<table>
<thead>
<tr>
<th>MMTV</th>
<th>Positive</th>
<th>Negative</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>≤56</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>&gt;56</td>
<td>4</td>
<td>46</td>
</tr>
<tr>
<td>Luminal A</td>
<td>ER+/PR+</td>
<td>5</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>HER2-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal B</td>
<td>ER+/PR+</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>HER+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER+</td>
<td>ER-/PR- &amp; HER+</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>TNBC</td>
<td>ER-/PR-</td>
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<td>10</td>
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<tr>
<td></td>
<td>HER2-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stage I</td>
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<td>45</td>
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<tr>
<td></td>
<td>Stage II</td>
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<td>17</td>
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<tr>
<td></td>
<td>Stage III</td>
<td>1</td>
<td>14</td>
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<td></td>
<td>Stage IV</td>
<td>4</td>
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<tr>
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<td>1</td>
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<td>Grade</td>
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<tr>
<td></td>
<td>Grade 3</td>
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<td>35</td>
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</table>
3.3. Clinicopathological Correlations in BLV and MMTV Positive Samples

An analytical assessment was performed to evaluate the association between each viral infection and the clinicopathological characteristics of the 103 tumor samples. In individuals aged ≤56 years, BLV was the most frequently detected, with 12 positive cases compared with three for MMTV. Conversely, among those aged >56 years, seven positive cases were observed for BLV and four for MMTV, respectively.

Regarding molecular subtyping, 11 luminal A samples tested positive for BLV viral infection, while five were positive for MMTV. In contrast, among luminal B cases, seven samples were positive for BLV and only two for MMTV. All six cases of HER2+ were negative for both viruses, and only one case among the TNBC cohort tested positive for BLV, with none observed for MMTV.

In the context of clinical staging, BLV positivity was observed in 12 stage I cases compared with only one case of MMTV. In stage II samples, only one sample tested positive for both BLV and MMTV. Furthermore, two patient samples exhibited positivity for BLV, while one case was positive for MMTV among stage III tumor samples. Finally, both BLV and MMTV were positive in four samples at stage IV.

Among the subset of tumor samples exhibiting lymphovascular invasion, BLV tested positive in 12 samples, whereas only six samples were positive for MMTV. Conversely, among samples without lymphovascular invasion, seven cases were positive for BLV compared with a single case for MMTV. In terms of lymph node involvement, 14 cases tested positive for BLV, while five were positive for MMTV. On the other hand, among cases without lymph node involvement, five and two cases were positive for BLV and MMTV, respectively.

We investigated the relationship between tumor size and the presence of BLV and MMTV positivity. By utilizing the greatest dimension as the cutoff point, we categorized samples into small (≤3 cm) or large (>3 cm) tumors, corresponding to T1/T2 and T3/T4, respectively. Our analysis revealed that the majority of BLV-positive samples (n = 12) were found among the small tumors, contrasting with the 43 samples that tested negative for BLV. Among the large tumors, only seven cases tested positive for BLV, while 41 were negative (Table 1). A similar trend was observed for MMTV positivity: out of the seven positive cases, three were among the small tumor groups (compared with 50 negative cases), and four cases were MMTV-positive among the 46 large tumor samples (Table 2).

Subsequent analysis considering the smoking history of BC patients was conducted. Among the 14 cases with a smoking history, only two samples tested positive for both BLV and MMTV, while the remaining 12 cases tested negative for both viruses. Conversely, among the nonsmoking patients, 17 tumors were found to be positive for BLV, while 72 were negative for the same virus. Regarding MMTV, five cases tested positive for the virus among patients with no history of smoking.

We finally assessed the relationship of family history to BLV positivity, which indicated that three of the cases that tested positive for BLV had a family history of BC diagnosis, whereas 16 cases were positive for BLV but lacked any family history (Table 1). A similar pattern was observed among the MMTV samples: one patient with a family history...
tested positive for MMTV, while five patients tested positive for MMTV without any family history (Table 2).

Unfortunately, a statistically significant association was not observed between BLV and MMTV status and any of the clinicopathological variables studied.

4. Discussion

BC presents a significant challenge to public health. Ongoing research exploring potential connections between BC and viral agents has suggested a potential role for BLV in promoting abnormal cell growth [24]. BLV DNA has been identified not only in healthy mammary tissue but also, and more frequently, in premalignant and malignant mammary tissue [29]. Moreover, antibodies to BLV capsid protein (p24) have been found in human blood and in non-small cell lung cancer [20].

However, in comparison to classical risk factors linked with BC, such as lifestyle, hormone replacement therapy, and reproductive history, the association between BLV and cancer appears equally significant [30,31]. In this context, the connection between BLV and cancer ranks second only to ionizing radiation and genetic factors [22].

In our study, the average age of patients with BC carrying BLV was 53.5 years old. However, we found that the difference was not statistically significant. This observation could be attributed to the relatively small sample size \( n = 103 \) evaluated in our study group.

BLV DNA was found in 18.4% of mammary tissue samples that exhibited histological signs of BC malignancy. None of the nonmalignant samples were positive for BLV. Our study did not include histological premalignant lesions. These findings are consistent with a previous study in Egypt, where BLV positivity was 16.0% [32]. However, conflicting results have arisen from different studies. While some reports have found no evidence of BLV DNA in mammary tissue, with or without carcinoma [27,28,33], other studies in Pakistan, Brazil, Iran, Columbia, and Australia have reported a significant correlation between BLV DNA and BC [21,29,34–36]. These discrepancies may arise from variations in the genetic backgrounds of the studied populations or differences in the methodologies used to detect viral genomes.

Research on premalignant lesions of the breast has revealed varying degrees of involvement [37,38]. This outcome was anticipated, given the extended latency period of factors implicated in breast carcinogenesis, which frequently precedes clinical detection. Additionally, BLV, a deltaretrovirus closely linked with HTLV-1, may persist within cell nuclei without integrating into the genome during asymptomatic and premalignant stages of leukemia/lymphoma [39]. During these stages, the virus is mediated by the nucleotide excision repair system. As a result, throughout the infected individual’s lifespan, DNA repair mechanisms become unstable, leading to genomic instability and the potential for neoplastic transformation [40].

The transmission mechanisms of BLV to humans have not been extensively explored, but they are speculated to be linked to the consumption of beef and milk products derived from infected animals [41]. Countries with lower consumption of these products reported lower rates compared with countries like the US and Australia, where the daily intake of calories derived from beef and dairy products is significantly higher [21,29,32,35–37].

Our study also explored the correlation between BLV and the molecular subtypes of BC. Most positive cases were reported among luminal A and luminal B patients. However, due to the limited number of patients positive and negative for the HER2 oncoprotein (HER2 subtype), this correlation did not reach statistical significance.

Other secondary factors examined for correlation included tumor stage, grade, the presence of metastatic tissue in lymph nodes, tumor size, smoking, and family history. The likelihood of metastatic tissue in sentinel lymph nodes increases with tumor size. The majority of our patients had tumors of <5 cm, along with a high number of patients with
tumor grades 2 and 3. These characteristics collectively indicate a poor prognosis. However, no significant correlation was observed with the presence of BLV DNA, likely due to the limited number of samples evaluated.

The detection of MMTV envelope sequence varies across different regions of the world, with reported prevalence rates ranging from 0% to 76% [42–46]. The underlying reasons for this geographical variation, as well as the role of MMTV in BC and its mode of transmission, are not fully understood. However, accumulating evidence suggests an association between MMTV and increased risk of BC.

A recent systematic review revealed that the presence of MMTV-like virus is associated with an increased risk of developing BC, with significantly higher prevalence rates observed in Western countries compared with Asian countries [47]. Furthermore, MMTV-like sequences of the envelope gene have been identified in breast samples of Australian women up to ten years before the development of BC and in BC patients, suggesting a potential causal association between the virus and BC [14].

It is believed that MMTV may have entered the human population through zoonotic transmission from mice in ancient times and has since been maintained through specific routes of transmission [48]. Two primary routes of transmission documented in the literature include saliva and milk [49]. Studies have detected MMTV DNA in the saliva of BC patients, healthy adults, and children, although not in newborns [50]. Moreover, MMTV sequences have been found in the milk of healthy lactating women, as well as in lactating women at high risk of developing BC, indicating another possible route of MMTV transmission among humans [49]. Overall, these findings underscore the need for further research to elucidate the mechanisms of MMTV transmission and its role in BC development, particularly in different geographic regions.

The pathogenesis of MMTV in humans appears to extend beyond BC, with studies reporting its presence in liver disease biopsies and its association with primary biliary cirrhosis, autoimmune liver diseases, and idiopathic liver diseases [51].

In this study, we detected the presence of MMTV envelope sequences in BC tissues in Jordanian women. The prevalence of MMTV-like envelope proviral sequences was found to be 6.8% among our BC cohort. Importantly, MMTV was not detectable in breast tissue from nonmalignant lesions included in the study. These findings align with the relatively low prevalence of MMTV reported in other countries, such as Saudi Arabia, Qatar, Tunisia, Jordan, and China [19,44,52,53]. However, it is notable that the prevalence of MMTV positivity in Jordan appears to be lower compared with studies from countries like Egypt, Italy, Sudan, and Morocco [45,46,54–56].

Overall, this study sheds light on the prevalence of MMTV in BC patients in Jordan and the Middle East region, contributing to our understanding of the geographic distribution of this virus and its potential role in BC development. Further research is needed to elucidate the factors influencing MMTV prevalence in different populations and to explore its implications for BC prevention and management.

No significant association was found between MMTV positivity and patient age, tumor grade, tumor stage, tumor size, lymph node involvement, smoking, and family history. Likewise, no significant association was found between the different molecular subtypes of BC and MMTV positivity. However, it is noteworthy that MMTV positivity was higher in patients with more advanced disease (grades 2 and 3) compared with those with grade 1 tumors. These findings provide valuable insights into the potential role of MMTV in BC progression and metastasis, warranting further investigation into the mechanisms underlying this association.

The findings of this study contribute to the growing body of evidence supporting the association between MMTV-like viruses and human BC, particularly in advanced tumors. The absence of a significant association between viral infection and patient age or tumor grade suggests that other factors may contribute to BC development in this population.
While the prevalence of MMTV positivity in this study was relatively low at 6.8%, it underscores the need for further investigation to fully elucidate the role of MMTV in BC etiology.

One limitation of this study is the relatively smaller sample size, which may affect the generalizability of our findings. While the results provide valuable insights, a larger sample size would enable more robust statistical analyses and strengthen the validity of our conclusions. In future research, we aim to increase the sample size to enhance the reliability of our results and to explore additional variables that may influence the outcomes.

5. Conclusions

In conclusion, this is the first study in Jordan that provides evidence supporting the association of BLV with breast cancer among Jordanian women. Prospective studies with larger sample sizes may be required to strengthen our current findings and conclusively establish BLV and MMTV as potential zoonotic agents with substantial public health implications.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres15020060/s1, Figure S1: RT-PCR amplification cycle graph displaying all positive MMTV samples among women with breast cancer. P: positive control; N: negative control; 4,13, 34, 37, 38, and 44 represent MMTV positive sample numbers.; Figure S2. Representative nested PCR bands of breast cancer samples in BLV positive and negative cases. L: 50bp ladder; N: negative control; P: positive control; 1, 2, 5, and 7 represent BLV negative samples; 3, 4, 6, and 8 represent BLV positive samples. Table S1: Raw Ct values for MMTV in BC samples from SYBR Green RT-PCR run. N: negative control; P: positive control.


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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of The Hashemite University (No.7/14/2020/2021) and KAUH (No. 42/151/2022).

Informed Consent Statement: Obtaining informed consent for this work was waived by both IRB protocols since all the samples used for this work were surplus (archived) breast tumor tissue samples, and patients undergoing surgery or biopsy collection provide informed consent to donate any excess tissue (i.e., beyond that needed for diagnostic purposes).

Data Availability Statement: The data presented in this study are available from corresponding authors upon request.

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