Molecular Detection and Oncogenic Association of Human Herpesvirus 8 (HHV-8) in Breast Cancer: Evidence from a Case Control Study in Iran

Ali Ramezani[1], Azarakhsh Azaran[1], Habibollah Mirzaei[1], Shahram Jalilian[1]*,Roya Pirmoradi[1], Shahram Bagheri[2], Zahra Khoramkouhi[1], Armin Mohammadi[3]

1. Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Department of Pathology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3. Department of Surgery, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

First Authors Ali Ramezani: https://orcid.org/0000-0003-1731-2767

Corresponding author*: Shahram Jalilian (PhD), Associate professor of medical virology at Ahvaz Jundishapur University of Medical Sciences

Address: Golestan Blvd., Esfand St., Ahvaz Jundishapur University of Medical Sciences, Department of Medical Virology, Ahvaz, 1579461357, Khuzestan province, Iran. Cancer Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

https://orcid.org/0000-0002-1827-2990

Tel: +989038470332 Email**:** norovirus2009@gmail.com

Abstract

Introduction: Breast cancer remains the most prevalent malignancy among women globally. The oncogenic role of human herpesvirus 8 (HHV-8) in breast pathology remains controversial and underexplored. Existing literature offers conflicting data on HHV-8 prevalence in breast tissue, and there is a paucity of research correlating viral presence with specific histological subtypes, such as ductal carcinoma in situ (DCIS), or molecular biomarkers within the Iranian population. This study investigates the "hit-and-run" viral hypothesis by comparing viral prevalence between pre-invasive (DCIS) and invasive (IDC) stages, while also providing a phylogenetic analysis of Iranian HHV-8 strains.

Objective: This study aimed to detect HHV-8 DNA in breast tissue samples and evaluate its association with malignancy, tumor grade, and clinicopathological characteristics.

Materials and Methods: A case-control study was conducted using 201 formalin-fixed, paraffin-embedded (FFPE) tissue blocks (110 malignant, 91 benign). HHV-8 DNA was identified via nested PCR targeting the *ORF26* gene, followed by sequencing and phylogenetic tree construction.

Results: HHV-8 DNA was significantly more frequent in malignant tissues (7.27%) than in benign lesions (1.10%; $p = 0.038$). Notably, viral prevalence was higher in pre-invasive DCIS (22.22%) than in invasive carcinoma (4.55%). HHV-8-positive cases exhibited elevated lymph node involvement (62.5%). Phylogenetic analysis clustered the Iranian sequences with East Asian strains.

Conclusion: HHV-8 is significantly associated with breast malignancy, particularly in the pre-invasive stage, which supports a transient oncogenic role. The virus correlates with aggressive clinical features, suggesting it may influence tumor progression during early carcinogenesis via inflammatory pathways and immune evasion mechanisms.

Keywords: Breast Neoplasms; Human Herpesvirus 8; Oncogenic Viruses; Phylogeny; Polymerase Chain Reaction

Running Title: HHV-8 infection linked to breast cancer risk

1. Introduction

Breast cancer (BC) is the most commonly diagnosed cancer in women worldwide. Its incidence and mortality rates have increased over the past three decades, driven by advances in cancer detection, better cancer registration and the profile of risk factors. Approximately 80% of BC patients are now people >50 years of age. Histologically, breast cancer is classified into Invasive Breast Carcinoma of No Special Type (NST) (formerly known as Invasive Ductal Carcinoma (IDC)) which accounts for 40–80% of cases, along with approximately 25% of special histological subtypes (e.g., invasive lobular, tubular, and mucinous carcinoma). Molecularly, based on mRNA gene expression levels, it is divided into intrinsic subtypes including Luminal A, Luminal B, HER2-enriched, and Basal-like, as confirmed by The Cancer Genome Atlas Project. [1]. In 2022, BC caused 2.3 million new cases and 670,000 deaths worldwide. While mortality is falling in high-income countries, it is still rising elsewhere. By 2050, the number of cases and deaths could rise by 38 % and 68 % respectively, with the impact in low-income countries being the most severe. Better early detection, treatment and data are needed to reduce the burden of disease [2]. In Iran, BC accounts for 76% of the most common cancers in women [3]. Carcinomas are cancerous tumors that originate from epithelial cells and account for the majority of BCs. The exact cause of BC is still unknown in many cases. Malignant breast transformation arises through a complex process involving hormonal, genetic, and environmental factors, including viral agents that disrupt

cellular pathways and drive uncontrolled growth [4]. BC has been reported in association with mouse mammary tumour virus (MMTV), high-risk human papillomaviruses (HPV), Epstein–Barr virus (EBV), and bovine leukemia virus (BLV) [5]. HHV-8, known as Kaposi's sarcoma-associated herpesvirus (KHSV), is a double-stranded DNA virus that belongs to the Herpesviridae family, subfamily Gammaherpesvirinae. Like the majority of viruses in this family, it spreads through bodily fluids like saliva [6]. The virus, first identified in Kaposi's sarcoma of an HIV-positive patient, was later confirmed as its cause and is also associated with multifocal Castleman disease and primary effusion B-cell lymphoma. Mechanistic studies of HHV-8 in virus-associated malignancies such as Kaposi sarcoma and primary effusion lymphoma have further elucidated the oncogenic role of latent genes (LANA-1, v-cyclin, vFLIP) and the secretion of viral interleukin-6, which activate survival and proliferation pathways [7]. A viral homolog of interleukin-6 (vIL-6) is encoded by HHV-8. The epithelial-to-mesenchymal transition (EMT), a hallmark of cancer invasion and metastasis, is facilitated by elevated IL-6. EMT is the specific process by which normal epithelial cells lose their structural integrity and acquire the ability to migrate. The presence of HHV-8 demonstrated a statistically significant association with estrogen receptor (ER) positivity, which is a significant finding that clarifies the oncogenic mechanism. This implies that the virus probably works with hormonal signaling pathways to promote the growth of tumors in breast epithelial cells.[8].

Like other herpesviruses, HHV-8 alternates between lifelong latency and lytic replication; during latency, episomal DNA expresses limited genes whose proteins promote tumorigenesis by enhancing angiogenesis, suppressing apoptosis, and stimulating proliferation [9]. The relationship between HHV-8 and BC has been the subject of relatively few studies worldwide, and even fewer in Iran. Therefore, it is critical to determine the relationship between this virus and BC. HHV-8 genome detection and genotyping were the objectives of this study, which was carried out in paraffin block samples of BC patients using the Nested-PCR method.

2. Materials and methods

2.1. Study population and sample collection

In the current case-control study, 91 formalin-fixed paraffin blocks (FFPE) of non-cancerous breast tissue and 110 paraffin blocks of BC were obtained from the pathology department of Imam

Khomeini Hospital in Ahvaz between 2020 and 2022. Following the receipt of the ethical code from the Jundishapur University of Medical Sciences in Ahvaz (IR.AJUMS.REC.1398.403), samples were collected under research project number OG-9819. First, using a microtome, 5–10 μm sections were cut from paraffin blocks and transferred into DNase- and RNase-free Eppendorf tubes. A pathologist reviewed hematoxylin and eosin (H&E)-stained sections to confirm the diagnosis and identify representative tissue areas. DNA extraction was then performed from the corresponding whole FFPE sections.

2.2. DNA extraction protocol

In order to extract DNA, 5–10 μm sections were first deparaffinized by incubating them with 1 ml of xylene at 37°C for an hour. They were then rinsed with ethanol at progressively lower concentrations (100, 70, and 50%) for 30 minutes. 300 μl of lysis buffer and 30 μl of proteinase K for lysis were added to each sample after the plates had been dried at 37°C. The samples were then incubated at 37°C for the entire night. Equal amounts of phenol, chloroform, and isoamyl alcohol were used to extract proteins from suspension, and absolute ethanol was used to precipitate the extracted DNA. Nanodrop evaluated the extracted DNA's concentration and purity. DNA extracted from FFPE blocks showed concentrations within an acceptable working range, an A260/280 ratio of approximately 1.8–2.0, and only samples with successful amplification of the 110 bp β-globin internal control were considered suitable for HHV-8 analysis.

Using the forward primer PCO3: 5'-ACACAACTGTGTTCACTAGC-3' and the reverse primer PCO4: 5'-CAACTTCATCCACGTTCACC-3', the 110 bp human globin gene product was amplified by PCR in order to evaluate the quality and integrity of the extracted DNA . β-globin PCR amplification was successful in all 201 FFPE samples included in the study; therefore, no sample was excluded from HHV-8 analysis because of inadequate DNA quality.

2.3. Detection of HHV-8

This study employed a nested polymerase chain reaction (nested-PCR) assay to detect HHV-8 DNA. Primers targeting the Open Reading Frame 26 (ORF 26) were utilized, with the outer primer set (5'-AGCCGAAAGGATTCCACCAT-3' and 5'- TCCGTGTTGTCTACGTCCAG-3') designed to amplify a 233 bp fragment. A subsequent round of amplification was performed using the inner primers (5'-TTCCACCATTGTGCTCGAAT-3' and 5'-TACGTCCAGACGATATGTGC-3'), which yield a 211 bp amplicon. The PCR products were visualized on a 2% agarose gel under UV transillumination. To confirm the results, the PCR was repeated, and only samples consistently positive were considered confirmed for HHV-8 presence [10]. Nested PCR amplification of HHV-8 ORF26 was performed in two rounds. The first-round PCR included an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 45 s, with a final extension at 72°C for 5 min. The second-round PCR was carried out using 1μL of the first-round product under the following conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s, with a final extension at 72°C for 5 min.

2.4.Sequencing and Phylogenetic analysis

SnapGene (GSL Biotech) software was used for analysis. Sequences were assigned accession numbers and registered in the GenBank database (XKQ45478.1). Using Mega 7.0 software, a phylogenetic tree was created by the Maximum Likelihood method after positive HHV-8 ORF 26 sequences were blasted in the web program (www.ncbi.nlm.nih.gov/BLAST).

2.5. Statistical Results and Data Presentation

The current study evaluates the prevalence of HHV-8 DNA in a clinical cohort comprising 110 patients with malignant breast diagnoses and 91 patients with benign breast lesions. Statistical analyses were performed using SPSS version 26; categorical variables were compared using the Chi-square test or Fisher's exact test when appropriate, continuous variables using the independent-samples t-test, odds ratios (ORs) with 95% confidence intervals were calculated, and a two-sided p-value < 0.05 was considered statistically significant. A multivariate analysis was referred to in the assessment of nodal involvement within the malignant subgroup, although the full model specification was not detailed in the manuscript.

3. Results

3.1. Demographic and Clinical Characteristics of the Study Population

The demographic analysis (Table 1) reveals a distinct age-related profile that aligns with established risk factors for breast malignancy. The malignant cohort (n=110) presented with a significantly higher mean age compared to the benign group (n=91), indicating that the window of oncogenic transformation or the manifestation of viral-induced changes correlates with advancing age.

Table 1: Clinical and demographic features of the research population

Feature	Malignant Cohort (n=110)	Benign Cohort (n=91)	Total (n=201)	Statistical Significance (p)
Age Analysis				< 0.001
Mean Age (± SD) [1]	53.42 ±13.84	36.16 ±11.52	45.62 ±15.21	
Age Range	29 – 84	14 – 75	14 – 84	
HHV-8 Detection				0.038
Positive Cases	8 (7.27%)	1 (1.10%)	9 (4.48%)	
Negative Cases	102 (92.73%)	90 (98.90%)	192 (95.52%)	
Primary Diagnosis				
Invasive Ductal Carcinoma (IDC)	88 (80.0%)	0 (0.0%)	88 (43.8%)	
Ductal Carcinoma in Situ (DCIS)	18 (16.4%)	0 (0.0%)	18 (9.0%)	
Other Malignant[2]	4 (3.6%)	0 (0.0%)	4 (2.0%)	
Fibroadenoma	0 (0.0%)	55 (60.4%)	55 (27.4%)	

Fibrocystic Changes	0 (0.0%)	19 (20.9%)	19 (9.5%)
Other Benign[3]	0 (0.0%)	17 (18.7%)	17 (8.5%)

[1]The malignant and benign groups were not age-matched, and age-adjusted logistic regression was not performed; therefore, age may have acted as a confounding factor in the observed association.[2]Other Malignant includes Lobular, Metaplastic, and Tubular carcinomas. [3]Other Benign includes codes for specific lesions and non-specified benign findings.

Statistical evaluation confirms that the presence of the HHV-8 genome is not randomly distributed; the malignancy group is roughly seven times more likely to harbor HHV-8 DNA than the benign group ($p = 0.038$), suggesting a potential association between the virus and the malignant state.

3.2. Distribution of HHV-8 Across Malignant Subtypes and Tumor Grades

A critical component of this analysis is the evaluation of viral prevalence within specific histological subtypes and stages of malignancy. The malignant cohort was primarily composed of Invasive Ductal Carcinoma (IDC) and Ductal Carcinoma in Situ (DCIS).

Table 2: HHV-8 Distribution in Malignant Subtypes and Tumor Grades

Variable	Total Cases	HHV-8 Positive (n)	Prevalence Rate (%)
Histological Classification			
Ductal Carcinoma in Situ (DCIS)	18	4	22.22%
Invasive Ductal Carcinoma (IDC)	88	4	4.55%
Tumor Grading (Nottingham System)			
Grade 1 (Well-Differentiated)	21	1	4.76%
Grade 2 (Moderately Differentiated)	59	4	6.78%
Grade 3 (Poorly Differentiated)	30	3	10.00%

3.3. Correlation with Immunohistochemical Biomarkers

The molecular landscape of breast cancer is largely defined by the expression of Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER2). This study investigated whether HHV-8 infection preferentially targets or influences specific molecular subtypes.

Table 3: Immunohistochemical biomarker correlation

Biomarker Expression	HHV-8 Positive (n=8)	HHV-8 Negative (n=102)	p-value
Estrogen Receptor (ER)			0.442
Positive	3 (37.5%)	43 (42.2%)	
Negative	5 (62.5%)	59 (57.8%)	
Progesterone Receptor (PR)			0.389
Positive	4 (50.0%)	42 (41.2%)	
Negative	4 (50.0%)	60 (58.8%)	
HER2 Expression			0.612
Positive	1 (12.5%)	9 (8.8%)	
Negative	7 (87.5%)	80 (78.4%)	
Equivocal	0 (0.0%)	13 (12.8%)	

The data indicate that HHV-8 positive tumors exhibit a slightly higher frequency of PR positivity (50.0%) compared to the negative cohort (41.2%), though this difference was not statistically significant. No statistically significant association was observed between HHV-8 positivity and ER, PR, or HER2 status.

3.4. Evaluation of Invasiveness and Metastatic Risk Factors

To assess the impact of HHV-8 on tumor behavior, the study analyzed three indicators of local and regional spread: Perineural Invasion (PNI), Lymphovascular Invasion (LVI), and Lymph Node (LN) involvement.

Table 4: Assessment of Metastatic Risk and Invasiveness

Aggressiveness Indicator	HHV-8 Positive (n=8)	HHV-8 Negative (n=102)	Odds Ratio (95% CI)
Perineural Invasion (PNI)	3 (37.5%)	32 (31.4%)	1.31 (0.30 - 5.75)
Lymphovascular Invasion (LVI)	4 (50.0%)	39 (38.2%)	1.61 (0.39 - 6.63)
Lymph Node Involvement	5 (62.5%)	47 (46.1%)	1.95 (0.45 - 8.41)

For PNI, LVI, and lymph node involvement, the estimated odds ratios were above 1; however, all 95% confidence intervals crossed 1 (Table 4), indicating that none of these associations reached statistical significance. These findings should therefore be interpreted as exploratory.

3.5. Phylogenetic tree

In the presented phylogenetic tree (Figure 1), the sequence XKQ45478.1 ORF26 (minor capsid protein)-HHV8 from Iran, marked with a black square, clusters closely with AE092513.1 ORF26-HHV8 from Iran and ABS71116.1 ORF26-HHV8 from China. This tight clustering suggests a high degree of genetic similarity and close evolutionary relationship among these sequences.

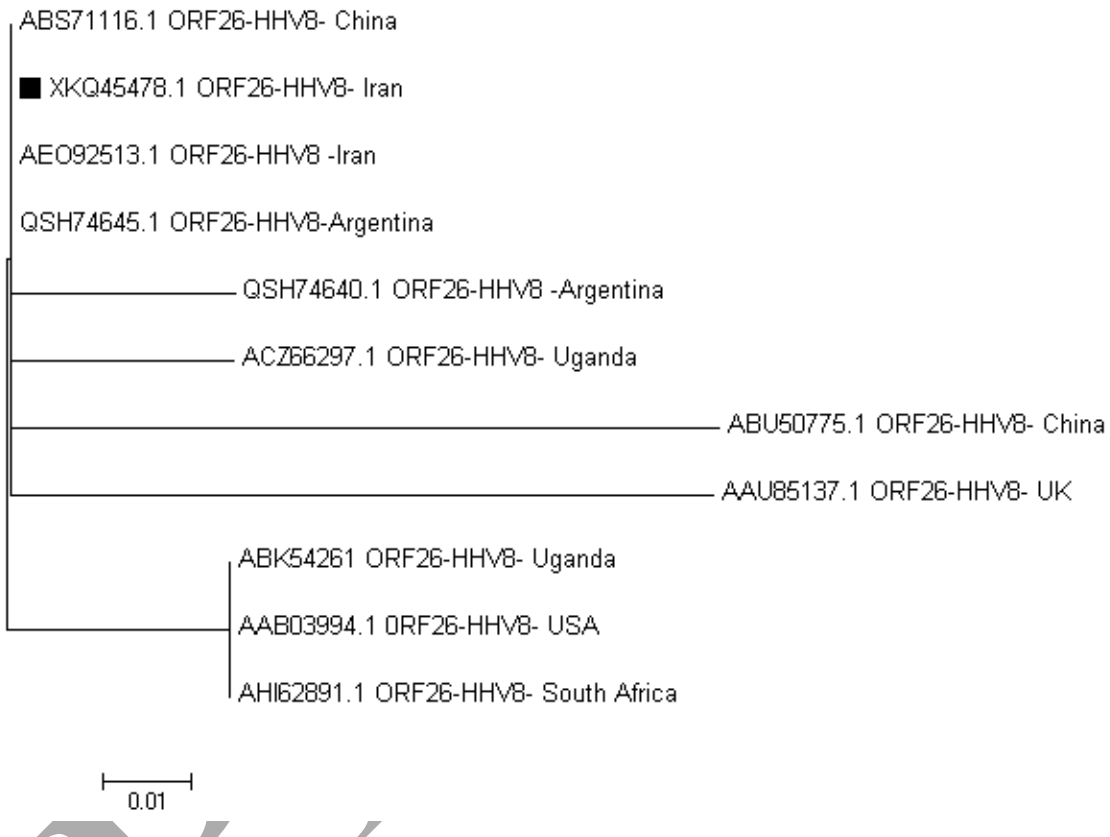

***Figure 1**: The evolutionary history was inferred using the Maximum Likelihood method in MEGA7*

4. Discussion

Finding HHV-8 DNA in breast cancer is a difficult scientific task that requires combining clinical pathology, epidemiology, and molecular biology. Discussing how this complex pathogen might interact with mammary epithelial cells is made possible by the current findings that demonstrate a significantly higher viral prevalence in malignant tissues, particularly in pre-invasive DCIS and high grade tumors.

HHV-8 is characterized by a distinct geographic distribution, which is reflected in the prevalence rates observed in breast cancer studies. High seroprevalence in regions such as sub-Saharan Africa and the Mediterranean contrasts with lower rates in North America and Northern Europe [17]. In the Iranian context, where this study was conducted, HHV-8 has been reported in varying percentages of breast cancer patients, ranging from approximately 12% in tissue samples to nearly 29% in blood samples [21].

The 7.27% tissue prevalence found in our malignant cohort is consistent with this regional baseline. The disparity between blood-based detection (high) and tissue-based detection (lower) suggests that HHV-8 might reside in circulating lymphocytes or monocytes that infiltrate the tumor microenvironment, rather than exclusively infecting the epithelial cells themselves. However, the strong association with malignant tissue over benign tissue ($p=0.038$) implies that whether the virus is within the tumor cell or its immediate environment, it contributes to an oncogenic milieu.

In line with reports that HHV-8 is enriched in more aggressive cancers, it is noteworthy that HHV-8-positive tumors were more frequently high grade and node-positive within the cancer group. As an example, A research conducted in Kirkuk city revealed that 76.47% of breast cancer patients who tested positive for HHV-8 had metastasis to adjacent lymph nodes, while only 23.53% of those without metastasis. In contrast, all breast cancer patients in this study who were HHV-8 negative did not exhibit metastasis [11].

However, when tumor grade was accounted for in multivariate analysis, grade (not viral status) was the sole independent predictor of nodal metastasis, implying that HHV-8 infection may be a marker of aggressive disease rather than a direct driver of metastasis in this cohort.

Results from earlier research on HHV-8 and breast cancer have been conflicting. KSHV/HHV-8 DNA was found in breast tumors at extremely high frequencies, according to some early PCR surveys. For example, Newton *et al.* (2003) detected HHV-8 in 55% of breast cancer samples [12], Tsai *et al.* found ~45% positivity [13], Liao *et al.* reported 87.5% of patients (versus 45% of controls) with HHV-8 [14], and Hsu *et al.* identified HHV-8 in 43.8% of cases [15]. By contrast, other investigations did not confirm such high rates. For instance, a PCR-based serological study in Iran found HHV-8 DNA in 21.3% of breast cancer patients and 18.8% of healthy women, a non-

significant difference [16]. Methodological and population differences, including tissue versus blood-based sampling, serological versus PCR-based detection, use of non-breast tissue populations, and geographic variation in HHV-8 prevalence, likely account for these discrepancies. Therefore, such studies are not directly comparable to FFPE breast tissue-based molecular analyses and should be interpreted cautiously. In contrast to some published reports, our malignant-tissue positivity (7.3 percent) was significantly higher than the 2 percent HHV-8 seroprevalence observed in healthy Iranian blood donors [17], highlighting an enrichment in tumor tissue. Collectively, these investigations imply that HHV-8 might infect a subset of breast cancers, although the actual frequency and significance of this infection differ depending on the study context.

A higher HHV-8 positivity rate was observed in DCIS (22.22%) than in IDC (4.55%). According to table 2, high prevalence in DCIS (22.22%) compared to IDC (4.55%) suggests that HHV-8 may play a transient "hit-and-run" role, perhaps facilitating the early disruption of the basement membrane before the viral load diminishes as the tumor achieves a state of independent invasive growth. Additionally, HHV-8 positivity was descriptively higher in Grade 3 tumors than in Grades 1 and 2; however, no formal trend test was performed, and given the small number of positive cases, this observation should be interpreted cautiously.

Biologically, HHV-8 (KSHV) is an established oncogenic herpesvirus that causes Kaposi's sarcoma, primary effusion lymphoma and Castleman's disease [18]. It is plausible that HHV-8 contributes to tumor biology in HHV-8–positive cancers. Our observation that HHV-8 positivity aligns with higher grade and nodal involvement is consistent with this idea. Moreover, a comprehensive review noted HHV-8's association with worse clinical outcomes in breast cancer: in one analysis HHV-8 had the stronges association with breast cancer of all tested viruses and was linked to both overall and relapse-free survival [13]. If HHV-8 infection does in fact encourage oncogenesis, this supports the more general idea of viral oncology, which holds that persistent infections have the ability to cause or hasten cancer.

Our phylogenetic findings further add context: Iranian HHV-8 sequences clustered with East Asian (notably Chinese) strains, suggesting regional patterns of transmission [19]. Similar analyses in

other Iranian cohorts have shown KSHV genotypes A/C prevalent in classic KS, aligning with Mediterranean and Asian lineages. This implies that HHV-8 reaching Iranian patients may reflect broader Eurasian viral circulation [20] The observed phylogenetic proximity may reflect a shared ancestral lineage or similar transmission routes of HHV-8 strains circulating in the Middle East and East Asia. This cluster diverges from sequences originating from other geographical regions, including Argentina, Uganda, the United Kingdom, the United States, and South Africa. Specifically, the Iranian and Chinese cluster branches off before the Argentinean clade (QSH74645.1 and QSH74640.1), which in turn is distinct from the Ugandan and other African and Western sequences. The increasing branch lengths and distinct nodes emphasize geographically structured genetic divergence. Of note, Ugandan sequences (ACZ66297.1 and ABK54261.1) appear in separate branches, indicating considerable intra-regional genetic diversity of HHV-8 strains within Uganda. Sequences from the United Kingdom (AAU85137.1), the United States (AAB03994.1), and South Africa (AHI62891.1) are situated on more distant branches, reflecting their independent evolutionary trajectories. The phylogenetic placement of XKQ45478.1 (Iran) within a tight cluster comprising another Iranian isolate and one from China supports the hypothesis of regional transmission patterns or historical epidemiological linkage. This lineage is phylogenetically distinct from HHV-8 sequences in sub-Saharan Africa, the Americas, and Europe, underscoring the geographical compartmentalization of HHV-8 genetic variants. These findings may provide valuable insights into the global distribution and molecular evolution of HHV-8.

The analysis of ER, PR, and HER2 biomarkers (Table 3) provides insights into the potential hormonal crosstalk between the virus and the host. Although statistical significance was not reached for biomarker correlation in our cohort, the literature suggests that viral proteins can bypass traditional hormone signaling [22]. For example, in other glandular cancers, HHV-8 has been shown to co-opt cellular signaling to allow for androgen-independent growth [23].

In breast cancer, the relative enrichment of HHV-8 in ER-negative and high grade tumors (10% in Grade 3) suggests that the virus may be more common in aggressive molecular subtypes, such as the triple-negative or HER2-enriched categories. This is consistent with findings that immune checkpoint markers like Herpesvirus Entry Mediator (HVEM) are upregulated in malignant breast tissue and associated with higher tumor grades and HER2 expression [24].

4.1. Limitations: Our study's small number of HHV-8-positive cases resulted in large confidence intervals and decreased statistical power. Because this was a retrospective, single-center analysis of FFPE specimens, there is potential for selection bias and for DNA degradation. Nested PCR, while sensitive, raises contamination concerns and detects only DNA presence (not viral activity). We did not assess HHV-8 gene expression or viral load, nor did we evaluate host immune status (e.g. HIV infection) or other risk factors that might confound the association. Finally, our control group consisted of benign breast lesions rather than normal tissue, which could influence baseline infection rates.

4.2. Future Directions: To fully understand HHV-8's role in breast cancer, more research is required. The presence of HHV-8 infection prior to tumor development may be ascertained through prospective cohort studies. Whether HHV-8 is active in tumor cells would be determined by studies of viral oncogene expression (for instance, by RT-PCR, in situ hybridization, or immunohistochemistry for latent/lytic proteins). Patients with HHV-8-positive cancers may have unique immunological signatures that can be identified through immunoprofile analysis (antibody titers, T-cell responses). It may be possible to more accurately estimate prevalence and account for confounders in larger, multi-center case-control or population-based studies. In conclusion, experimental research (e.g., A. KSHV infection of mammary epithelial cells) could be used to test causality. In summary, we recommend a combination of epidemiological, virological and immunological approaches to validate and extend these findings.

5. Conclusion

Although a number of studies have examined the connection between HHV-8 and breast cancer, the field is plagued by inconsistent prevalence reports worldwide. In particular, there aren't many tissue-based studies on the Iranian population; instead, the majority of the data are based on serological tests. Additionally, the lack of comparative data between DCIS and IDC makes it more difficult to comprehend how the virus contributes to the development of tumors. Lastly, it is challenging to link particular viral genotypes to carcinogenesis due to the scant phylogenetic information from breast-derived samples.

One of the most intriguing results from our study is the significantly higher prevalence of HHV-8 in DCIS (22.2%) compared to IDC (4.5%). DCIS represents a pre-invasive stage where the basement membrane remains intact. The sharp decline in viral detection as the tumor progresses

to the invasive IDC stage is highly characteristic of the "hit-and-run" hypothesis of viral carcinogenesis [25]. The phylogenetic clustering of Iranian HHV-8 strains with East Asian isolates highlights regional epidemiology and suggests possible transmission routes. More broadly, if a viral role in breast cancer is confirmed, it could inform diagnostics and prevention strategies: for example, anti-HHV-8 therapies or vaccines might eventually be considered as part of cancer control [15]. Regardless, our results highlight the significance of taking oncogenic viruses into account when studying tumor biology and public health.

Conflict of Interest: The authors hereby declare that there are no conflicts of interest with regards to the publication of this paper.

Declaration:

Ethical Approval: We would like to inform that this study has received approval from the Ethics Committee of AJUMS (IR.AJUMS.REC.1398.403).

Clinical Trial Number: Not applicable.

Consent to Participate: The study (OG-9819) was conducted using paraffin-embedded archival tissue samples, which are considered de-identified and do not require individual patient consent for participation.

Consent to Publish: All authors have approved the final manuscript and consented to its publication.

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Data Availability: The datasets generated and/or analyzed during the current study are not publicly available due to their confidential nature as they contain sensitive patient information from hospital archives. However, the coding data files and analysis results will be made available to the journal's editors and reviewers upon reasonable request.

Sequence data that support the findings of this study have been deposited in the NCBI (https://www.ncbi.nlm.nih.gov/protein/XKQ45478.1) with the Accession No : XKQ45478.1

Author Contribution: S.J., A.R. and **A.A.** wrote the main manuscript text. **H.M.** prepared the figure and Tables. **S.B.** conducted the analyses, and laboratory work was done by **R.P., A.M** and **Z.K.** All authors reviewed and approved the final manuscript.

Declaration Regarding the Use of AI in Research

In this study, the AI model (**Gemini pro 3.0**) was utilized for grammatical correction and improvement of English writing.

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