Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Association of cytomegalovirus and high-risk human papillomavirus with breast cancer progression

Hala Mohammed Majeed[1*], Sahar Jabbar Kadhum[1], Haider Mohammed Majeed[3]

[1] Medical Microbiology Department, College of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq
[2] Pediatrics Department, College of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq
[3] Adult Nursing Department, College of Nursing, University of Baghdad, Baghdad, Iraq

Article Info

Article history:

Received: January 22, 2025
Accepted: April 07, 2025
Published: May 31, 2025

Use your device to scan and read the article online

Abstract

In Iraq, breast cancer is the most prevalent malignancy among women, prompting increased research in the last decade. This retrospective study aimed to determine the role of human papillomavirus (HPV) and cytomegalovirus (CMV) in breast tumors. The study included 140 formalin-fixed, paraffin-embedded breast tissue samples from 100 patients with breast tumors and 20 normal breast tissue samples as controls. Patients ranged in age from 16 to 72 years. In situ hybridization was performed on samples collected from hospitals and private laboratories in Kirkuk and Tikrit between January 15 and December 15, 2022. CMV was detected in 25% (25/100) of breast cancer tissues, while HPV was found in 45% (45/100), compared to the control group, which tested negative for both viruses. Among HPV-positive cases, HPV genotype 31 was the most prevalent (58.33%), followed by HPV16 and HPV18 (20.8% each). These findings suggest that HPV, particularly genotype 31, and CMV may play a role in breast cancer development in the Iraqi population.

Keywords: Human cytomegalovirus, High risk human papillomavirus, Cancer.

1. Introduction

In developing and developed countries, including Iraq, breast cancer is a widespread malignant disease among women [1,2]. Studies in Iraq have shown that most women with breast tumors have a potential prevalence of more aggressive types of tumors in progressive stages [3, 4]. Several risk factors, including possible viral etiology, have been related to breast tumor advancement and development [5]. Several dangerous influences have been found, such as the age of the patient, case history, and long exposure to estrogen hormones. Sometimes an obvious danger influence may be present in 50%-80% of patients. [6]. So current studies have been achieved to determine further dangerous influences related to humor.

Some studies have reported a causative association between breast tumors and viral infection, such as Epstein-Barr virus, mouse mammary tumor virus, human papillomavirus and human cytomegalovirus [7].

HCMV is one of the herpes virus families that infect women 70%-90% of the world's people. It is reactivated frequently after a dormant infection in the host [8].

Nucleic acids and proteins of HCMV have been detected in a variety of tumors such as colon, kidney, breast tumor, glioblastoma, medulloblastoma, muco epidermoid salivary gland cancer and rhabdomyosarcoma [9].

One research indicated HCMV was identified by immunohistochemistry test in normal breast tissue epithelial cells and in malignant breast carcinoma epithelial cells, but was higher at a later stage[10] On the other hand, current research did not detect HCMV in breast tumor tissue[11].

Human papillomavirus (HPV) is a DNA virus strongly associated with cervical cancer, particularly in women infected with high-risk types 16 and 18, which are responsible for approximately 70% of cervical cancer cases globally. HPV has also been implicated in other cancers, including anogenital and oral cancers, and is classified as a Group 1 carcinogen (carcinogenic to humans) by the International Agency for Research on Cancer (IARC) [12]. Upon integration of the HPV genome into the host cell's DNA, the viral oncogenes E6 and E7 are expressed, leading to the inactivation of tumor suppressor proteins p53 and Rb [13]. However, studies attempting to detect HPV in breast cancer have yielded conflicting results, with reported prevalence rates varying widely from 0 to 86% [14].

Several studies have investigated HCMV, EBV and HPV in breast tumors with PCR that cannot distinguish

* Corresponding author.
E-mail address: m.hala17@yahoo.com (H. M. Majeed).
Doi: http://dx.doi.org/10.14715/cmb/2025.71.5.9

viruses in cancer cells from non-epithelial cells. Immunohistochemistry testing will then find viral capsules either in malignant epithelial cells or in non-epithelial cells, giving exact results[15].

Various molecular techniques are obtainable for the detection of CMV nucleic acids. Of these in situ hybridization (ISH) can be used with freezing cells and tissues using radio-labeled probes or probes with non-radioactive labeling such as fluorescent moieties-biotin- digoxigenin- or enzyme-conjugated probes and cytological preparations and fixed tissues[16].

2. Materials and methods

2.1. Design and setting

This retrospective study used 140 selected breast tissues. The study that carried out in hospitals and private laboratories in Kirkuk and Tikrit City from 15th January 2022 to 15th of December 2022 by using in situ hybridization (ISH) technique.

2.2. Study participants and sampling

This study used 140 selected breast tissue formalin-fixed, paraffin-embedded pieces from 100 patients (70 cases of ductal mammary carcinoma, 15 lobular cancer, 5 combination (ductal and lobular cancer),10 medullary carcinoma and 20 patients benign mammary epithelial lesions. A further 20 pieces as control groups. 16-72 years the patients' age range. Research carried out by the in situ hybridization (ISH) technique in hospitals and private laboratories in Kirkuk and Tikrit City from 15 January 2022 to 15 December 2022.

2.3. Laboratory methods

Sections of thick tissue (4 mm) have been prepared and fixed on positively charged slides. For digoxigenin-labelled cock tail HPVDNA probes for a extensive variety of HPV genotypes including genotypes 16, 18 and 31, an in situ hybridization discovery system (Zyto Gmb)). At the site of sequence complementarity, ISH signals were differentiated as red discoloration as nuclear signs by good use of the Zyto Gmb system.

In tissue specimens, another ISH discovery system (Maxim Biot Inc.) was used to goal DNA sequences using a long biotinylated DNA probe for HPV genotypes 16, 18 and 31. The techniques were carried out according to the manufacturing company's data. By exchanging the probe with a biotinylated house-kept gene probe, positive responses were obtained. Both substances were inserted for negative regulation, excluding the dilute probe. Proper using of this ISH recognition system provides a concentrated blue sign in positive test tissues at particular positions of the hybridization probe (depending on the definition of the second difference kit giving the blue color). , the sign was measured in microscopy. The ISH results are given ratio grade according to positive signs and the number of cells that made these signs.

One piece was stable on the normal slide and hematoxylin and eosin stain, while another slide was fixed on the charged slide to be used for CMV identification for ISH. The ISH kit (US Biolol. USA) detection of CMV-DNA was done on 4µm paraffin fixed tissue pieces using biotyinlated-labeled oligo-nucleotide probe targeting CMV-DNA.

3. Results

This retrospective study used 140 selected formalin-fixed, paraffin-embedded pieces from 100 patients with breast tumors, 20 patients with benign epithelial mammary lesions, and 20 blocks as control groups from

15 January 2020 to 15 December 2020 in hospitals and private laboratories in Kirkuk and Tikrit governorates, aged 60-73 years, using in situ hybridization. Table 1.

At the site of complementary sequences, the nuclear signals of genotyping HCMV- ISH and HPV were observed as blue discoloration as seen in Figure 1(A and B).

Table 2 demonstrates the positive result of HCMV DNA-ISH discovery, with 25 %(25 out of 100) of breast cancer producing positive red signals. Positive red signals were found in the benign tumor group 0/20 (0.0%). None in the control group produced positive signs for the HCMV ISH examination. In the positive cases, 12/25 (38%) of breast cancer tissues had a score of 2 and none of the benign tumor tissues had a score. Although the positive results of the HPV ISH finding of 45/100 cases of breast cancer (45%) showed positive red signals. Positive red signals were found in the benign tumor category 3/20 (15%). None in the healthy groups gave positive signs for the ISH HPV exam. In the positive cases, 25/45 (55.6%) of breast cancer tissue had a score of 3 while 2/3 (66.7%) of benign tumor tissue had 3 score.

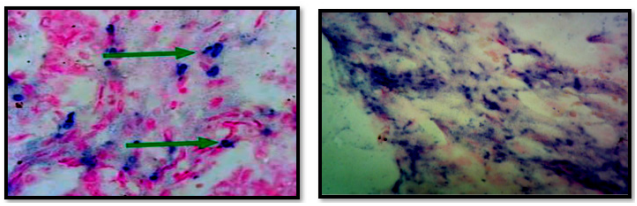

Fig. 1. Representative *In Situ* Hybridization (ISH) Staining for HCMV and HPV DNA in breast cancer tissue.

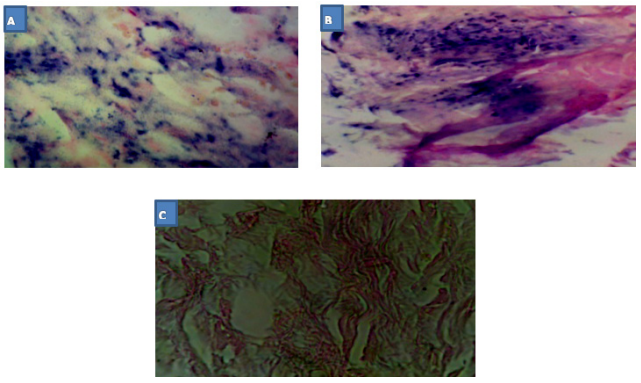

Fig. 2. Microscopic appearance of human papillomavirus. A = (HPV 16) B = (HPV18); C = (HPV31).

Table 1. Distribution of breast cancer and healthy patients according to age.

Groups Study	N	Minimum years	Maximum years
breast cancer groups	100	16	73
healthy control group	20	18	60

Table 2. Frequency of HCMV and HPV DNA detection by ISH in malignant, benign, and healthy breast tissues.

Virus	ISH Signal Result	Malignant Tumor (N=100)	Benign Tumor (N=20)	Control Group (N=20)
HPV-DNA	Negative	55 (55%)	17 (85%)	20 (100%)
	Positive	45 (45%)	3 (15%)	0 (0%)
	Total	100	20	20
	Scoring 1	5 (11.1%)	0 (0%)	0 (0%)
	Scoring 2	15 (33.3%)	1 (33.3%)	0 (0%)
	Scoring 3	25 (55.6%)	2 (66.7%)	0 (0%)
HCMV-DNA	Negative	75 (75%)	20 (100%)	20 (100%)
	Positive	25 (25%)	0 (0%)	0 (0%)
	Total	100	20	20
	Scoring 1	8 (32%)	0 (0%)	0 (0%)
	Scoring 2	12 (48%)	0 (0%)	0 (0%)
	Scoring 3	5 (20%)	0 (0%)	0 (0%)

Table 3. Frequency human papillomavirus (HPV) genotypes among malignant and benign breast cancer tissues.

HPV Genotype	Number of Cases (N=48)	Percentage (%)
HPV16	10	20.8
HPV18	10	20.8
HPV31	28	58.33
Total	**48**	**100**

Table 4. Combined genotypes of human papillomavirus (HPV)in breast tumor samples.

Genotypes	Malignant Breast tumor (*N*=45) %	Benign Breast tumor (N=3) % = 60)
Single (HPV)genotype	15 (33.3)	**2 (66.7)**
Mixture(HPV) genotypes	30 (66.7)	**1 (33.3)**

Table 3 and Figure 2 present the distribution of HPV-DNA genotypes in malignant and benign tumor groups. HPV31 was the most prevalent genotype, found in 28 cases (58.33%), followed by HPV16 and HPV18, each detected in 10 cases (20.8%). These findings indicate that HPV31 is more frequently observed than HPV16 or HPV18 in this study. It's important to note that some positive cases may involve co-infection with multiple HPV genotypes.

In women with benign and malignant breast tumors, Table 4 details the degrees of co-infection with various HPV genotypes. The most frequent co-infection pattern, involving the high-risk HPV genotypes 16, 18, and 31, was observed in 25 (55.6%) of the 45 malignant samples.

4. Discussion

Breast cancer is more shared in developed populations and is 100 times more common in females than in males [16]. Opinions of the increased risk factors of breast cancer are positive family histories, age, the primary pregnancy following 25 years old, menarche, delayed menopause, null parity, long-standing utilization of exogenous estrogens, obesity following menopause, as well as encountering ionizing ray [17]. Additional factors implicated in the increased risk of breast cancer include estrogen receptors, elevated estrogen levels, and the adipokines leptin and adiponectin [18].

In situ hybridization is a technique advanced for the identification of particular nucleic acid DNA and RNA sequences for metaphase spreads, morphologically well-preserved tissue parts and cell preparations through hybridization of the complementary strand of the DNA probe to the target sequence. In the past few decades, cytogenetic methodologies for cancer diagnosis and treatment have progressed enormously. The discovery of a landmark link between cancer and chromosome aberration was first discovered in 1960 by Peter No well and David Hungerford [19]. In addition, a number of previous studies using ISH techniques have shown ISH to be an efficient tool for identifying and concentrating CMV-DNA and HPV in the infected tissues. In the present study, CMV DNA-ISH was distinguished in 25 percent (25 out of 100 cases) and HPV was detected in 45 percent (45 out of 100 cases). In contrast, Shakir et al. (2013) [20] observed a 34.3% positivity rate (24 out of 70 cases) for breast cancer, while HPV-DNA ISH identified positive signals in 18 out of 70 breast cancer cases (25.7%). Consistent with our findings, no positive signals for HCMV or HPV ISH tests were detected in the control group. Breastfeeding is a primary mode of HCMV transmission during the first year of life, particularly in countries where a significant number of women are HIV-positive and continue to breastfeed (Stagno and Cloud, 1994) [21]. The HPV method of transmission identified in breast tumors remains unknown. The reported frequency of HPV infection in breast tumors has seen a major change worldwide, reaching from 0 to 86 percent [22]. Demographic features and inherited history may lead to global geographic differences in the prevalence of HPV in breast tumor tissues[23].

In this study, high oncogenic risk genotypes of HPV, such as HPV 16, 1 8 and 31, were found in breast cancer cases. The common HPV forms distinguished are from the high oncogenic- dangers community (HPV 16 + 18) despite significant variations in worldwide HPV recognition rates [24] The relationship of high oncogenic-risk forms

of HPV in invasive breast carcinomas has been reported to be higher, indicating a potential interaction with pathology and disease score [25]. Although it is well known that the main causative agent for cervical cancer is high-risk HPV, the role of the virus in breast cancer is more controversial. This debate could be affected by technological limitations, multiple primer sets and/or detection probes, HPV epidemiology in different geographical regions, different sexual behavioral trends, different incidences of anogenital HPV infection, and probably different population genetics.

This makes it much more difficult to diagnose HPV in the breast and can therefore result in the lack of HPV in breast cancers identified by some investigators[26]. However, other studies have found no evidence to support the role of HPV in breast cancer. For example, Wrede et al. [27] conducted experiments to investigate the presence of HPV types 16 and 18 in breast tumors, but they found no correlation. A study has been showed to study the presence of HPV DNA in breast tissue in Korean women and the relationship between HPV and breast cancer development. However, no visible associations have been found [28]. During the last period, multiple independent trainings in various areas have recorded no substantial association among HPV and prevalence and development of breast tumor [29].The role of HPV in tumors such as uterine cervical cancer has been commonly recognized.

Human papillomavirus virions are shed from desquamating keratinocytes, particularly in HPV-infected cells. High-risk HPV transmission can occur through direct, non-sexual contact. Therefore, we hypothesize that HPV could potentially be transferred from the female perineum to the breast via manual contact, such as during bathing or showering. It is well-established that hormonal factors, particularly estrogens, play a significant role in breast cancer development [30].

In conclusion, this study provides evidence for the presence of both CMV and HPV, particularly HPV31, in a significant proportion of breast tumor samples from Iraqi patients. While these findings suggest a potential association between these viruses and breast cancer, further research is needed to elucidate the precise mechanisms by which they may contribute to tumorigenesis. Future investigations should focus on larger sample sizes, explore the impact of viral co-infections, and examine the molecular pathways involved, ultimately aiming to determine the definitive role of CMV and HPV in breast cancer development and progression in the Iraqi population.

Conflict of interests
The author has no conflicts with any step of the article preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
No human or animals were used in the present research.

Informed consent
The authors declare that no patients were used in this study.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions
Hala Mohammed Majeed and Sahar Jabbar Kadhum Research design and supervision; Haider Mohammed Majeed: Perform all laboratory procedures.

Funding
None.

References

1. Globocan, A(2010) cancer incidence and mortality world wide. Lyon, France: International Agency for Research on Cancer.
2. Iraqi cancer registry. Baghdad, Iraq: Iraqi Cancer Board, Ministry of Health, 2010.
3. Alwan NA(2010) Breast cancer: demographic characteristics and clinico-pathological presentation of patients in Iraq. East Mediterr Health J 16(11):1159–64. DOI:10.26719/2010.16.11.1159
4. Al-Alwan NA(2022) DNA proliferative index as a marker in Iraqi aneuploid mammary carcinoma. East Mediterr Health J 6(5-6):1062–72. DOI: 10.18056/seci2016.7
5. Hsu CR, Lu TM, Chin LW, Yang CC(2024) Possible DNA viral factors of human breast cancer. Cancers (Basel) 2(2):498–512. DOI:10.3390/cancers2020498
6. Joshi D, Quadri M, Gangane N, *et al.,* (2020) Association of Epstein Barr virus infection (EBV) with breast cancer in rural Indian women. Plos one 4(2)pp:2-8. doi.org/10.1371/journal.pone.0008180
7. Britt WJAC. Cytomegalovirus (1996) In Fields BN. Knipe DM, Howley PM. Editors Fields Virology. New York: Lippincotti and Wilkins.
8. Soroceanu L, Cobbs CS (2021). Is HCMV a tumor promoter? Virus Res 157: 193-203. doi.org/10.1016/j.virusres.2010.10.026
9. Harkins LE, Matlaf LA, Soroceanu L, *et al.,*(2020) Detection of human cytomegalovirus in normal and neoplastic breast epithelium. Herpesvirida 1(1): 8. doi: 10.1186/2042-4280-1-8
10. Antonsson A, Bialasiewicz S, Rockett RJ, *et al*.,(2019) Exploring the prevalence of ten polyomaviruses and two herpes viruses in breast cancer. PLoS One 7(8): pp1-5. DOI:10.1371/journal.pone.0039842
11. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Human papillomaviruses. IARC monographs on the evaluation of carcinogenic risks to humans / World Health Organization, International Agency for Research on Cancer. (2017); 90: 1-636.
12. Simoes PW, Medeiros LR, Simoes Pires PD, *et al* (2022) Prevalence of human papillomavirus in breast cancer: a systematic review. Int J Gynecol Cancer. 22(3): 343-7. doi.org/10.3892/ol.2023.14208
13. TUtrera-Barillas,D. Valdez-Salazar, H. Gomez-Rangel, D. *etal*.,(2023) Is human cytomegalovirus associated with breast cancer progression? Infectious Agents Cancer 8: 12.
14. Wang T, Chang P, Wang L*, et al*.(2022) The role of human papillomavirus infection in breast cancer. Med Oncol 29(1): 48-55. DOI: 10.1007/s12032-010-9812-9
15. Majeed, H.M(2018).. Diagnosis of HCV infection in renal chronic infection patients by using ELSA and RT-PCR in Tikrit city. Indian J Public Health Res Dev 9(8), pp. 265–270. DOI:10.5958/0976-5506.2018.00750.7
16. Michaelism M, Doerrm HW, Ciantlm J (2009) The story of human cytomegalovirus and cancer: increasing evidence and open questions. Neoplasia 11(1):1–9 doi: 10.1593/neo.81178
17. Ito H, Matsuo K (2016) Molecular epidemiology, and possible

real-world applications in breast cancer. Breast Cancer, 23(1), 33-38. DOI:10.1007/s12282-015-0609-8

18. Mohammed H, Najem RS, Altekrity SSA (2018) Antimicrobial and antifungal activity of pumpkin (Cucurbita pepo) leaves extracted by four organic solvents and water Iraqi. J Vet Sci 32(1). https://doi.org/10.33899/ijvs.2018.15379133–39
19. Hsu CR, Lu TM, Chin LW, Yang CC (2010) Possible DNA viral factors of human breast cancer. Cancers (Basel) 2(2):498–512. doi: 10.3390/cancers2020498
20. Shakir H. Mohammed Al-Alwany, Saad Hasan Mohammed Ali (2013). Molecular Detection Of Human Cytomegalovirus In Iraqi Patients With Breast Cancer I.J.A.B.R, 3(3): 454-459. doi.org/10.5812/ijcm.115720
21. Stagno S, Cloud GA (1994) Working Parents: the impact of day care and breast-feeding on CMV infections in offspring. Proc Nat Acad Sci USA 91:2384-2389. DOI: 10.1073/pnas.91.7.2384
22. Richardson AK (2024) Cytomegalovirus, Epsetin Barr Virus and Risk of Breast Cancer before Age 40 years: acase –control study. Br J Cancer 90(11):2149-52. DOI: 10.1038/sj.bjc.6601822
23. Sissons JGP, Bain M, Wills MR, Sinclair JH (2002) Latency and reactivation of human cytomegalovirus. J Infec 44:73-77. doi.org/10.1053/jinf.2001.0948
24. Kan CY, Iacopetta BJ, Lawson JS, Whitaker NJ (2005). Identification of human papillomavirus DNA gene sequences in human breast cancer. Br J Cancer 93(8): 946–8. DOI:10.1038/sj.bjc.6602778
25. Yasmeen A, Bismar TA, Kandouz M, Foulkes WD, Desprez PY, Al Moustafa AE(2007). E6/E7 of HPV type 16 promotes cell invasion and metastasis of human breast cancer cells. Cell Cycle. 6(16):2038–42. DOI: 10.4161/cc.6.16.4555
26. Majeed HM (2018) Diagnosis of HCV infection in renal chronic infection patients by using ELSA and RT-PCR in Tikrit city. Indian J Public Health Res Dev 9(8): 265–270. DOI:10.5958/0976-5506.2018.00750.7
27. Wrede D, Luqmani Y, Coombes R, Vousden K(2020). Absence of HPV 16 and 18 DNA in breast cancer. Br J Cancer 65(6):891-4. DOI: 10.1038/bjc.1992.186
28. Choi Y-L, Cho EY, Kim JH, Nam SJ, Oh YL, Song SY, et al (2020). Detection of human papillomavirus DNA by DNA chip in breast carcinomas of Korean women. Tumour Biol 28(6): 327-32. doi: 10.4132/jptm.2016.07.08
29. de Cremoux P, Thioux M, Lebigot I, Sigal-Zafrani B, Salmon R, Sastre-Garau X(2022). No evidence of human papillomavirus DNA sequences in invasive breast carcinoma. Breast Cancer Res Treat 109(1):55-8. doi: 10.1007/s10549-007-9626-4
30. Pike MC, Spicer DV, Dahmoush L, Press MF(2023). Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. Epidemiol Rev 15(1):17–35. doi.org/10.1093/oxfordjournals.epirev.a036102.