International Journal of Advanced Engineering Research and Science (IJAERS)
Peer-Reviewed Journal
ISSN: 2349-6495(P) | 2456-1908(O)
Vol-10, Issue-1; Jan, 2023
Journal Home Page Available: https://ijaers.com/
Article DOI: https://dx.doi.org/10.22161/ijaers.101.14

Influence of Oncoproteins E6 and E7 of high-risk Human Papilloma Virus on Breast Cancer

Ana Karina Brizeno Ferreira Lopes[1,2,3,4,5,6*], Marina Viana Padilha[5], Julliano Matheus de Lima Maux[5], Sandra Maria Souza da Silva[1,5], Elayne Interaminense Cavalcanti de Brito Azevedo[5], Eloiza Maria do Nascimento[5], Júlia Barroso Cirne de Azevedo[5], Cristiane Moutinho Lagos de Melo[4], Jacinto Costa Silva Neto[5]

[1]Hospital das Clínicas da Universidade Federal de Pernambuco, Brasil
[2]Hospital Universitário Oswaldo Cruz, Brasil
[3]Hospital de Câncer de Pernambuco, Brasil
[4]Programa de Pós-Graduação em Biologia Aplicada à Saúde da Universidade Federal de Pernambuco – Laboratório de Imunopatologia Keizo Asami (LIKA), Brasil
[5]Laboratório de Pesquisa Citológicas e Molecular da Universidade Federal de Pernambuco, Brasil
[6]Centro de Diagnóstico Boris Berestein – Diagnóstico da América (DASA)

Received: 12 Dec 2022,
Receive in revised form: 09 Jan 2023,
Accepted: 16 Jan 2023,
Available online: 29 Jan 2023

***Keywords*— Breast cancer, E6, E7, HPV, Oncogene proteins**

***Abstract*—** *Breast cancer is the most frequent neoplasm in the world, and its causes are multifactorial. Some risk factors are already well established, and others are still being studied, such as infections with certain agents, like the human papillomavirus (HPV). This study aimed to carry out a literature review about the association between breast cancer and HPV, aiming to analyze the role of their E6 and E7 oncoproteins, using PUBMED and LILACS databases for the research. Of the 41 articles included in the study, 30 endorse the association of HPV with breast cancer, of these, only eight with statistically significant values. This study found HPV 16 as the genotype with the highest detection rate and ductal carcinoma as the histological pattern with the highest frequency. Formalin-fixed and paraffin-embedded tissue (FFPE) was the most studied type of sample storage and preservation, and polymerase chain reaction (PCR) was the most used detection method. The virus genetic material was found integrated into the host cell genome in most of the samples. Given the findings, it was concluded that HPV can have a great influence on breast carcinogenesis, however, studies involving the carcinogenic pathways of HPV, and morphological and molecular patterns should be carried out involving a larger sample.*

I. INTRODUCTION

Breast cancer has the greatest incidence in the world, among all the types of cancer, with approximately 2.3 million new cases per year, representing 11.7% of new cancer diagnoses worldwide[1]. It is also the most frequent cancer in women, corresponding to 24.2% of neoplasms in this group[2]. It is one of the main causes of cancer deaths in women in both developed and developing countries[3].

Breast cancer has multifactorial causes. Female gender, early menarche, late menopause [4], aging, family history of breast cancer, nulliparity, first pregnancy after the age of 30, high breast tissue density, excessive alcohol consumption, physical inactivity, overweight, exposure to ionizing radiation[5], use of hormonal contraceptives, post-menopausal hormone therapy, smoking[6], breast cancer gene 1 and 2 (BRCA1 and BRCA2) mutations, and ovarian

cancer in the family[2] are among the most well-established risk factors.

Age over 50 years is considered the most important risk factor[2]. The incidence increases with age, doubling the risk every 10 years until menopause[7]. Other factors, however, may be associated with a lower risk of developing this cancer, such as breastfeeding, adequate physical activity, a regular healthy diet, normal body weight[5], and early pregnancy[4].

In addition to the well-established risk factors for breast cancer, infectious agents may represent a new factor that plays a key role as carcinogens or cancer promoters [3]. Several viruses have been documented to have oncogenic potential, including mouse mammary tumor virus (MMTV); bovine leukemia virus (BLV); Epstein-Barr virus (EBV), also known as human herpes virus type 4; and human papillomavirus (HPV)[4].

HPV DNA has already been found in breast tumors in several studies involving several countries [8–11]. In addition to the genome, koilocytes, and oncoproteins E6 and E7 were also found in breast cancer [4].

The evidence on the role of HPV in breast cancer is substantial, but not conclusive [4]. This study aims to gather information about the possible etiological association of HPV and its E6 and E7 oncoproteins in breast carcinogenesis.

1.1 BREAST CANCER

There are more than 20 different histological patterns of this disease, with about 80% being represented by ductal carcinoma[5]. Other frequent carcinoma subtypes are lobular, tubular, mucinous, medullary, micropapillary, papillary, and cribriform[5,12]. They can be further categorized according to the degree of infiltration in the breast tissue, in situ or invasive; and subclassified by the degree of tissue differentiation based on the level of nuclear pleomorphism, the mitotic index, and glandular and tubular formation, in well-differentiated (grade 1), moderately differentiated (grade 2) and poorly differentiated (grade 3)[12].

Molecular classification, on the other hand, better assesses the risk of tumor recurrence and progression, allowing the selection of the best therapy and estimating the final prognosis[12]. The most widely used markers, especially in patients with invasive carcinoma, are estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor type 2 (HER2), and the mutation in the p53 tumor suppressor gene[12].

There are currently four known classical molecular patterns: Luminal A, the most common, corresponding to about 40% of cases, represented by positive ER and/or PR, negative HER2, and low proliferation rate (reduced Ki67); Luminal B, the second most frequent, with 20% of the neoplasms, characterized by positive ER and/or PR, HER2 negative and high Ki67; Overexpression of HER2, corresponding for 10-15% of tumors and characterized by positive and overexpressed HER2, and ER and PR generally negative; Basaloid, representing 15-20% of carcinomas, also known as triple negative (TN), that is, ER, PR and HER2 negative, with a lower survival rate (TABLE 1). Other patterns still mentioned are the Breast-normal simile and Claudin-low, corresponding to 12-14% of cancers [12,13].

Table 1. Classical molecular patterns of breast cancer

MOLECULAR PATTERNS	FREQUENCY	ER	PR	HER2	Ki67
Luminal A	40%	+	+	-	< 20%
Luminal B	20%	+	+	-	> 20%
Overexpression of HER2	10-15%	-	-	+	
Basaloid	15-20%	-	-	-	

ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth fator type 2

1.2 HUMAN PAPILLOMAVIRUS - HPV

The Papilloma Virus (PV) is a virus formed by a small double strand of circular, encapsulated DNA, composed of eight genes [14]. It has a tropism for cutaneous or mucocutaneous epithelium and uses the host's enzymatic mechanism to replicate its genome[15]. The Human Papillomavirus (HPV) comprehends a total of 40 different viral genotypes that are infections in humans, although several others animals species, such as birds, reptiles, marsupials, and other mammals are also infected by PV [14,15].

The HPV genome measures eight kilobases (kb) in length and is formed by eight open reading frames (ORF)[6]. It is divided into three segments: long control

region (LCR), early region (E), and late region (L)[3]. The LCR region represents 10% of the genome and regulates E6 and E7 transcription; E is formed by six genes, known as E1, E2, E4, E5, E6, and E7, responsible for encoding proteins involved in viral replication, transcription, and transformation; and L encodes the structural proteins, L1 and L2[3,6].

There are approximately 200 HPV subtypes listed [16]. An HPV is classified into a new lineage when its nucleotide sequence in the L1 ORF gene differs by at least 10% from any other type already characterized [14,15].

The alphapapillomavirus genus (alpha HPV) is detected in the mucosal epithelium and is related to the development of benign and malignant diseases, such as cervical, anal, and head and neck cancer[15].

HPV can be subdivided into high-risk (hr HPV) or low-risk (lr HPV), depending on its propensity to develop malignant lesions[6].

In cervical cancer, the most oncogenic subtypes, hr HPV, are alpha groups 5, 6, 7, and 9, responsible for about 90% of all cervical neoplasms in the world. The most common sublines in this cancer are: alpha 9 – HPV 16, 31, 33, 35, 52, 58 and 67; alpha 7 – HPV 18, 39, 45, 59, 68, 70, 85 and 97, and alpha 10 – HPV 6 and 11. Among all the sublines, HPV 16 is the most involved with cervical cancer[15].

The natural course of cervical cancer development involves an initial sexual exposure to an oncogenic HPV, followed by the persistence of the virus and the development of a precursor intraepithelial lesion, subsequently evolving to an invasive lesion[15].

HPV in cervical cancer infects cells of the basal layer of the stratified squamous epithelium, through microcracks, and these cells, when generating mitosis, synthesize new viruses and produce daughter cells that can undergo modifications. The infected basal cell becomes a reservoir of infection, with the viral genome maintained at low copy numbers in the episomal form. After the virus accesses the cell, it integrates into the host genome and makes use of the cellular mechanisms of the host to replicate, causing high levels of copies in the upper layers of the epithelium. In this integration, the virus may lose part of its E2 gene, leading to deregulated expression of E6 and E7 proteins, which inhibit tumor suppressor proteins, such as p53 and retinoblastoma protein (pRB)[6].

E6 and E7 oncoproteins act as stimulators of host cell proliferation. E6 is an antagonist of the p53 and BCL2 gene (B-Cell Leukemia/Lymphoma 2), increasing chromosomal instability and cellular resistance to apoptosis; and E7 interacts with pRB releasing a transcription factor, called E2F, which promotes cell proliferation. E7 also upregulates S-phase genes and cyclin A and E, however, it also inhibits cyclin-dependent kinase inhibitors, such as WAF1 and kinesin-like protein (KIP 1), known as p21 and p27. The E6 and E7 proteins also interact with the BRCA1 and BRCA2 genes, antagonizing their functions, which prevent the development of tumors, by repairing DNA damage[3].

In addition to the association of HPV infection with cervical cancer, other tumors have also shown this relationship, such as anal canal and head and neck neoplasms, however, the involvement with breast lesions remains controversial[17].

1.3 HPV AND BREAST CANCER

Because Band, in 1991[18], identified that HPV 16 and 18 can immortalize mammary epithelial cells by inhibiting apoptosis, the detection of HPV in adenocarcinomas was observed in several tissue sites, alerting to the possibility that it is involved in the pathogenesis of some types of breast carcinomas[8,17,19].

The first time that HPV was observed in a breast tumor was in 1992, by Di Lonardo[20], when HPV 16 DNA was identified in 29.4% of malignant breast tissues embedded in paraffin using the technique of Polymerase Chain Reaction(PCR) with specific primers for HPV 11, 16 and 18[17].

The prevalence of HPV is significantly higher in breast cancer tissues compared to normal and benign breast tissues. Hr-HPV is four times more prevalent in breast tumors when compared to patients without cancer, and its infection is associated with an increased risk of developing this cancer by about 5.4 times. Hr-HPV was also identified in benign breast tissues, a finding that was associated with the emergence of HPV-positive breast cancer between 1 and 11 years later[4].

The prevalence of HPV positivity in breast cancer, as well as the HPV subtypes, varies greatly between countries and even between regions within the same country. For example, the rates of hr HPV associated with breast cancer in some Chinese Provinces are around 0-2%, while in North America is 86%[4].

II. METHODOLOGY

This study consists of an analytical literature review about the association between breast cancer and the HPV virus, aiming to analyze the role of the viral E6 and E7 oncoproteins.

Data collection was carried out from April 1st to April 30th, 2021, using the National Library of Medicine (PUBMED), Science Direct and Latin American and

Caribbean Literature on Health Sciences (LILACS) databases for the research.

Articles published in the last 10 years (between 2011 and 2021), were included. The descriptors "breast cancer", "HPV", "E6 and/or E7", their combinations and variants in English were used, with publications in Portuguese and English were sought.

As exclusion criteria, articles with an impact factor lower than 0.8 were removed from the study.

As a result, 41 articles were selected that fit the inclusion criteria and descriptors analyzed. After this selection, the following steps were followed: exploratory reading, selective reading and choice of material that fit the objectives and theme of this study, analytical reading and text analysis, and interpretive reading and writing.

III. RESULTS AND DISCUSSION

3.1 PERCENTAGES OF FREQUENCY OF HPV IN BREAST CANCER

Eight articles advocate for the association of HPV with breast cancer, with a detection of the virus in carcinoma tissues ranging from 7.5% to 95.5%, with statistical significance (TABLE 2)[3,8–10,21–24].

Table 2: Studies confirming the presence of HPV DNA in breast cancer samples (P value < 0.05).

Author/year	Tissue preservation	Detection means (primers)	Result	P value
Sigaroodi et al.,2012	FFPE	PCR (GP5+/GP6+, CP, and FAP)	15/79 (25.9%)	0,019
Piana et al., 2014	FFPE	PCR (SPF10)	6/80 (7.5%)	0,026
Fu et al., 2015	FFPE	PCR and ISH (HPV58 E7)	PCR: 25/169 (14.8%) ISH: 17/169 (10.06%)	PCR: 0.001 ISH: 0.008
Delgado-Garcia et al., 2017	FFPE	PCR (GP5+/GP6+), CLART, and DIRECT FLOW CHIP	130/251 (51.8%)	<0,001
Islam et al., 2017	Fresh frozen	PCR (MY09/MY11, LCR, and HPV16 E6-E7)	Pre-therapeutic = 174/272 (63.9%) After treatment w/ chemotherapy = 29/41 (71%)	0.001
Afshar et al., 2018	FFPE	RT-PCR (MY09/MY11)	8/98 (8.2%)	0,051
Cavalcante et al., 2018	FFPE	PCR (MY09/MY11, GP5+/GP6+) mPCR (E6 and E7)	51/103 (49.5%)	<0,0001
Khodabandehlou et al., 2019	Liquid nitrogen (-80ºC)	PCR (L1 and E7)	35/72 (48.6%)	0,003

bp= base pairs, FFPE = formalin-fixed and paraffin-embedded tissue, PCR= Polymerase chain reaction, ISH = in situ hybridization, LCR = long control region, RT-PCR = Real-time PCR

GP5+/GP6+: L1 specific primers (150bp)/ SPF10: L1 specific primers (65bp)/ MY09/MY11: L1 specific primers (450bp)

Some articles also show evidence of association and/or do not deny exclusion of association, however, they present a non-significant or unreported p-value. The detection rate ranged from 10% to 79.3% (TABLE 3)[7,13,25–43].

Table 3: Studies that defend the association of HPV with breast cancer and/or do not deny exclusion of association, but with non-statistical or unreported p values.

Author/year	Tissue preservation	Detection means (primers and bp)	Result	P value
Herrera-Goepfert et al., 2011	FFPE	PCR (SPF10 and HPV 18 and 33 E6) e RT-PCR (HPV 16)	6/60 (10%)	NR
Glenn et al., 2012	Fresh frozen	PCR (HPV 16, 18 and 33 E6)	10/27 (37%)	NR

Herrera-Goepfert et al., 2013	FFPE	PCR (LIC1/LIC2, MY09/MY11, GP5+/GP6+ and HPV 16 and 18 E6).	8/20 (40%)	NR
Suarez et al., 2013	Fresh frozen	PCR (MY09/MY11 and GP5+/GP6+)	16/61 (26%)	NR
Ahangar-Oskouee et al., 2014	FFPE	Nested PCR (MY09/MY11 and GP5+/GP6)	22/65 (33,8%)	NR
Corbex et al., 2014	FFPE	TS-MPG: mPCR and Luminex (specific primers)	15/123 (12,19%)	NR
Ting Wang et al., 2014	Liquid nitrogen (-80ºC)	HPV capture with MPS	1/7	NR
Ohba et al., 2014	Fresh frozen	DNA Chip technology – DNA microarray (TOSHIBA)	65/210 (31%)	NR
Banerjee et al., 2015	FFPE	PathoChip and PCR (E2, E4, L1 and L2)	79/100 (79,3%)	NR
Yan Chen et al., 2016	FFPE	Dual-PCR (HPV 16 and 18 E6 and E7)	HPV18E7 18/76(23.68%) HPV18E6 5/76(6.58%) HPV16 E6 and E7 0/76(0%)	NR
Ilahi et al., 2016	FFPE	PCR (GP5+/GP6+) TS (HPV 16 and 18)	HPV16 8/46 (17.3%) HPV18 0/46 (0%)	NR
Wang et al., 2016	FFPE	HIS	52/146 (35.6%)	NR
Naushad et al., 2017	FFPE	Standard PCR (GP5+/GP6+)	45/250 (18.1%)	NR
Salman et al., 2017	Fresh frozen	PCR and Imunnoblotting (*western blot* and *dot blot* for E7)	35/74 (47%)	NR
Wang, Y. X. et al., 2017	FFPE	qPCR (HPV 16 E6)	14/50 (28%)	NR
Wang Y. W. et al., 2017	Fresh	HC2 assay	14/81 (17.3%)	NR
Elamrani et al., 2018	Frozen	PCR TS-MPG	19/76 (25%)	0,28
Habyarimana et al., 2018	FFPE	PCR (GP5+/GP6+, MY09/MY11)	22/47(46.81%)	NR
Carolis et al.,2019	FFPE	HIS and PCR (MY09/MY11 and HPV 16 E6)	83/273 (30.4%)	NR
Sher et al., 2020	Fresh	PCR	5/50 (10%)	NR
El-Sheikh et al., 2021	Frozen	RT-PCR (E1, E2, E6, and E7)	16/72 (22.2%)	NR
Mofrad et al., 2021	FFPE	PCR (MY09/MY11, GP5+/GP6+, HPV 18 E6 and HPV 16 E7)	7/59 (11.8%)	NR

bp= base pairs, FFPE = formalin-fixed and paraffin-embedded tissue, PCR= Polymerase chain reaction, NR = Not reported, TS-MPG = bead-based multiplex genotyping (combine multiplex PCR and bead-base Luminex technology) , MPS =

massive paralleled sequencing, mPCR = multiplex PCR, HIS = in situ hybridization, qPCR = real-time quantitative PCR, HC2 = hybrid capture, RT-PCR = Real-time PCR

GP5+/GP6+: L1 specific primers (150bp)/ SPF10: L1 specific primers (65bp)/ MY09/MY11: L1 specific primers (450bp)

Eleven articles reported finding no association between breast malignancy and HPV[19,44–53]. Of these, five had a 0% rate of finding [45–48,52]. Some, however, observed some degree of detection, although in low percentages, ranging from 1.6% to 16%([19,44,49–51,53](TABLE 4).

Table 4: Studies that do not support the association between HPV and breast cancer.

Author/year	Tissue preservation	Detection means (primers and bp)	Result	P value
Aguayo et al., 2011	FFPE	PCR (SPF10 and HPV 16 E6 and E7)	L1 4/46 (8.7%) E6 and E7 0/3 (0%)	NR
Hedau et al., 2011	Fresh frozen	PCR and RT-PCR	0/228 (0%)	NR
Chang et al., 2012	Fresh	FQ-PCR (HPV 6, 11, 16, and 18 E6 and E7) and HIS	0/48 (0%)	NR
Herrera-Romano et al., 2012	FFPE and Fresh	PCR (GPE5+/GP6+ and HPV 16 E6)	0/118 (0%)	NR
Junping Peng et al., 2014	Liquid nitrogen (-80ºC)	PCR	2/100 (2%)	NR
Gannon et al., 2015	Fresh frozen	PCR (GP5+/GP6+ and MY09/MY11)	13/80 (16%)	0,6072
Li et al., 2015	FFPE	Nested PCR (GP5+/GP6+, MY09/MY11, and E6 and E7)	3/187 (1,6%)	NR
Karimi et al., 2016	FFPE	PCR (GP5+/GP6+ and E7)	2/70 (2,56%)	0,496
Bakhtiyrizadeh et al., 2017	FFPE	PCR (GP5+/GP6+ and MY09/MY11)	0/150 (0%)	NR
Ngamkham et al., 2017	Fresh frozen	PCR (GP5+/GP6+)	15/350 (4.285%)	NR
Kouloura et al., 2018	Preservative liquid (ThinPrep®)	PapilloCheck® genotyping assay (DNA chip)	0/201 (0%)	NR

FFPE = formalin fixed and paraffin-embedded tissue, PCR= Polymerase chain reaction, bp= base pairs, NR = Not reported, RT-PCR = Real-time PCR, FQ-PCR = real time fluorescence quantitative PCR, HIS = in situ hybridization

GP5+/GP6+: L1 specific primers (150bp)/ SPF10: L1 specific primers (65bp)/ MY09/MY11: L1 specific primers (450bp)

3.2 HPV SUBTYPES AND BREAST CANCER

In this study, we also sought to correlate the detection method used with the detection rate of viral histological subtypes. Among articles with statistical significance [3,8–10,21–24], viral detection for high-risk subtypes varied between 0.6%, referring to the detection rate of HPV 31 and 33, in the study by Afshar et al., 2018[24], to 69%, referring to HPV 16, by Islam et al., 2017[9]. HPV 16 also had the highest average detection rate in this review, appearing in 34.23% of the samples analyzed in the articles studied[3,8–10,21,22,24]. The higher detection of HPV subtype 16 found is an expected result, given its oncogenic potential observed in other malignant tumors caused by HPV[54].

It is important to note that both the study that presented the lowest detection rate of HPV 16, corresponding to

0%[10] and the one that presented the highest, referring to the rate of 69%[9], used PCR with primers for L1 gene and specific primers for E6 and/or E7 oncoproteins, suggesting that the difference in results was not influenced by the detection method. It is also worth noting that most samples (78.4%) from Cavalcante et al., 2018[10], did not identify any specific viral genotype.

HPV 16 was also the most prevalent viral subtype among studies that did not obtain a statistically significant value and/or did not include a "p-value" in the article but did not deny the association between the virus and breast carcinogenesis[7,13,25,27,28,30,31,33–35,37–43] with an average rate of 30.34%. Despite finding a rate of 10% of HPV 16 in their samples, the study by Herrera-Goepfert et al., 2011[35], concluded as insufficient to indicate an association. Ahangar-Oskouee et al, 2014[40], found 26.5% of HPV 6 and 1.5% of HPV 16, 35, 52, and 11 in Iran; since most samples that tested positive showed low-risk genotypes for malignancy, it was not possible to conclude a causal relationship between the variables. Furthermore, this study did not use primers to search for E6 and/or E7 proteins, only for L1, which may have contributed to the results obtained and the conclusions drawn about it.

In this review, eleven studies deny or did not find an association between HPV and breast cancer [23,44–53]. Of these, seven[19,44–48,53] attempted to detect HPV 16, eight[19,45–47,49–51,53] HPV 18, and four[19,45,46,53] the low-risk genotypes, 6 and 11. One study[52] did not specify the genotypes investigated. Kouloura et al., 2018[45], in addition to the subtypes mentioned above, also analyzed the presence of another 20 genotypes, including HPV 31 and 33, also high-risk. The detection methods used by these studies were predominantly the same used by studies with evidence of viral presence, in this case, it does not seem that there was a relationship between the method and the absence of detection.

These results support the hypothesis that HPV may have a role in breast carcinogenesis. Among the articles that showed an association or did not deny the possibility of its existence, genotypes 16 and 18 were the most prevalent, in agreement with the literature[15].

3.3 HISTOLOGICAL AND MOLECULAR PATTERNS OF BREAST CANCER AND HPV

Ductal carcinoma was the most frequently found histological subtype in breast cancer samples with HPV DNA detection [3,7,19,21,26,29,30,32–36,39,40,44,50,53,55], especially the invasive pattern (ICD)[7,19,21,26,30,32–35,39,40,50,53,55]. This percentage ranged from 45.7%[3] to 100%[26,32,35,36,44].

The second most frequent histological pattern in some reports was the lobular pattern [3,21,30,33,40], ranging from 9.52%[40] to 33,3%[33]. Ohba et al. 2014[43] found, however, a higher frequency of this pattern with 10/11 positives (90,9%), while the ductal was positive in 99/191(51,83%) (p=0.047).

HPV was also detected in other histological types of cancer, such as tubular[3,39], medullary[3,43], metaplastic[7], mammary[7], mucinous[35,43,53], papillary[35,39] and Phyllodes[40].

Some authors have not found HPV positivity in some histological patterns of carcinoma such as invasive lobular[26,32,34,44,52], mucinous[3,39,40,44], medullary[21], ductal in situ[21,53], adenocystic, apocrine, comedocarcinoma[39], Phyllodes[32]. All these studies, however, had the same limitation, the small sampling of these histological types, which may explain the absence of detection observed. The low viral load detected in several studies could also explain the low frequency of HPV detection in some samples[22].

This higher prevalence of HPV in the ICD raises the hypothesis that the gateway to HPV infection is due to the exposure of the mammary ducts to the external environment, and therefore, most breast cancers originate from this epithelium [30,34,36]. Also, it is believed that this virus could interact or act to initiate the development of cancer or increase the progression of the lesion in the presence of other cofactors[34].

Despite this predominance in the ICD, no significant association between the histological patterns of breast cancer and HPV infection, however, was found among the articles[3,21,33,35,39,48,53,55].

Other studies found significant HPV detection results when compared to breast cancer samples with normal or benign breast tissue, however, the histological categories were not specified[8–10,24,37].

Naushad et al. 2017[29], Aguayo et al. 2011[44], and Habyarimana et al. 2018[33], in turn, observed a greater detection of HPV infection in patients with more advanced breast cancer, with lymph node metastatic involvement, although not statistically significant, as did Mofrad et al. 2021[36], in which all HPV-positive cases were high-grade tumors. Glenn et al. 2012[37], Ilahi et al. 2016[27], Naushad et al. 2017[29], and Habyarimana et al. 2018[33] found no significance between tumor grade and HPV positivity in samples with cancer; as well as, Suarez et al. 2013[39], Ngamkham et al. 2017[53] and Delgado-Garcia et al, 2017[8], when they evaluated the lymph node status; and Delgado-García et al. 2017[8] and Habyarimana et al. 2018[33], with the clinical stage.

Few studies have compared the detection of HPV with hormone receptors, such as RE, RP, HER, p53, and Ki67,

and most have not found statistical relevance[8,29,33,35,37,39,43,50,53,55], however, some samples were small, which causes this limitation[33,37].

Ohba et al. 2014[43], however, noted that ER-positive breast cancer had a statistically higher HPV prevalence than ER-negative ones (p=0.0378), as Ilahi et al. 2016[27] for HPV 16 in hormone receptor-positive breast cancer (ER/RP and HER-2 positive) (p=0,032). Habyarimana et al. 2018[33] also observed greater detection in luminal A pattern (57.14%, 12/21), when compared with luminal B (33.33%, 3/10), TN (44.4%, 4/9) and HER2 overexpression (50%, 3/6); already Delgado-Gárcia et al. 2017[8] found a greater association with negative luminal B/HER2 immunophenotype, but both with non-significant p. In luminal subtypes, in A, HPV DNA was proportionally related to lymph node invasion (p=0.0007); in B, it was associated with high levels of Ki67 expression, that is, with a higher rate of cell proliferation, which infers greater aggressiveness (p=0,0188)[13].

Hormonal factors are involved in breast carcinogenesis, and HPV is a hormone-responsive virus, which has increased replication in the presence of steroid hormones. Its E6 and E7 oncoproteins, together with the action of estrogens cause cervical cancer, and this may be one of the roles of HPV in hormone receptor-positive breast cancer, but the association between HPV infection and ER expression is still controversial[33].

Piana et al. 2014[22], however, compared 40 samples of the TN phenotype with non-TN, finding HPV DNA only in the first group, with statistical relevance, suggesting a potential etiopathogenic role in this poorly differentiated tumor type (15%, p=0.026). Herrera-Goepfert et al. 2013, identified a viral DNA rate of 40% in samples of TN metaplastic carcinomas, [38]. Carolis et al. 2019[13] identified a statistical predominance in breast cancer with aggressive characteristics, the TN subtype (12/27.44%) and HER2+ (15/31.48.4%), comparing to Luminal A (34/142, 23.9%) and Luminal B (22/73.30.1%) (p=0.0181). Proteins E6 and E7 collaborate with HER2/neu, inducing breast tumorigenesis, in addition to acting in the metastatic process[33], which could explain the findings of these studies.

3.4 SAMPLE TYPES FOR RESEARCH FOR HPV IN BREAST CANCER

The studies in this research used different forms of breast tissue samples for HPV research: formalin-fixed and paraffin-embedded (FFPE), fresh tissue, and frozen tissue.

Six of the eight analyzes that showed a statistically significant association of the virus with carcinogenesis used FFPE samples[10,21–24], with HPV detection rates ranging from 7.5% to 51.8% (mean of 26.28% considering detections by PCR). Islam et al., 2017[9] used fresh frozen tissue, finding 63.9% of viral detection in pre-chemotherapy tumors, and 71%, in post-chemotherapy. Khodabandehlou et al.,2019[3] used liquid nitrogen at -80o, with detection of 48.6% among samples with cancer.

The largest sample of this review is that of studies that advocate for/do not deny the association between the variables but did not obtain or did not report results of statistical significance. Among these, FFPE was the most used sample type [13,25–29,31,33,35,36,38,40,41] with an average detection rate of 29.76%. Seven studies used fresh frozen tissue [7,30,32,34,37,39,43] and obtained a mean detection rate of 28.31%. Only the study by Ting Wang et al., 2014[42] used liquid nitrogen, detecting 14.28% of viral presence.

The study described by Chang et al., 2012 (42), analyzing fresh samples, found the presence of viral genetic material in 10% of benign lesions, but without evidence of infection in malignant lesions. This study also verified that the samples initially positive were later negative in a period of 3 months of freezing at -70ºC. This finding raises the importance of using fresh samples, as they are more sensitive than paraffinized samples.

Of the studies that achieved no degree of detection, three used fresh frozen tissue [46–48]. The study by Hedau et al., 2011[47], together with Bakhtiyrizadeh et al., 2017[52], also used FFPE. Only one [49] used liquid nitrogen and one [45] used liquid medium (Thin Prep®).

It was not possible to define the best way of tissue sample/storage in this review since the detection averages were very close between the different media and most articles used FFPE.

3.5 HPV DETECTION METHODS IN BREAST CANCER

The HPV viral load in breast cancer is extremely low compared to cervical cancer (approximately two thousand times lower) [4]. As a result, techniques used to detect the virus may present divergences in expressing them [56]. Factors involved in the detection of HPV DNA in breast samples such as the type of study performed, sensitivity of the molecular analysis method, histological type of the tumor, quality and integrity of the extracted DNA, conservation of samples, genotypic variation, population group evaluated and non-use of good laboratory practices with compliance with protocols, influence the results found[17,31,33].

Inadequacies in the screening and identification processes of the HPV virus can compromise clinical screening of oncogenic types of the virus [57]. Studies show that the viral DNA used for PCR may be subject to

degradation by contamination [17]. Minimizing dubious responses to viral presence, using PCR solutions controlled for DNA quality through a test with proven accuracy prevents false negatives [7,57].

The presence of HPV DNA in samples of benign and malignant breast lesions using quantitative real-time PCR (qPCR) and primers for regions E6 and E7 identified high-risk human papillomavirus, with a higher prevalence of hr HPV genotypes 16, 18, and 33. Of the 72 tumors selected, 35 were HPV positive, and 30 (86%) had the physical state of the integrated HPV[7]. Some authors consider the viral load together with the persistence of the infection a predictive factor for the genesis of the cervical lesion[23,38]. Based on this information, determining the viral load and the physical status of the virus in studies involving the association of HPV positivity and breast carcinogenesis becomes important.

More studies show the presence of oncogenic HPV DNA, such as 16, 18, 31, 33, 35, 45, 52, and 58. There were variations in the percentages of hr HPV from 1% to 51.8%[8,19,23,33].

Some analyzes did not confirm the presence of the virus in the tumor samples evaluated [45,47,48], therefore, the etiological role of HPV in breast carcinogenesis remains inconclusive [58]. Kouloura et al. 2018[45] performed a Microarray assay with 201 patients who had breast cancer, to detect HPV DNA of 24 types. Despite the high sensitivity of the technique used, no HPV DNA was identified in the samples evaluated, however, some characteristics of breast tumors were different among patients with HPV DNA in their cervical samples.

In cervical cancer, the interactions between HPV and carcinogenesis of the cervix are already consolidated[59], however, the pathways of interactions between HPV and breast cancer continue to need clarification [6]. An important condition is that even in studies with positive results, the subtypes detected tend to be diverse [8,19,23,33,42].

Virus amplification by PCR was the most used technique for HPV detection, presenting results that range from positive [3,8–10,21–24], statistically not relevant [7,13,25–27,29–41] and negative [19,44,46–53]. The use of genomic sequencing [42], in situ hybridization [46], hybrid capture [55], and immunohistochemistry (IHC)[31], are also capable of detecting HPV, however, they present controversial results compared to other studies[45].

Studies that employ the use of PCR using primers GP5+/6+, MY09/11, or SPF10 for amplification of the L1 region, can detect a greater number of genotypes. However, methodologies that only look for the presence of L1 are susceptible to negative results, since, when the viral genome is integrated, the L1 structural protein is absent because the viral capsid is lost in the integration[34,60,61], resulting in an unreliable outcome. Therefore, it is necessary that researchers also use specific primers for HPV proteins with oncogenic potentials such as E6 and E7, whose presence suggests greater viral activity[62,63], so that their findings can support that the oncogenic activity of HPV has functional aptitude in breast carcinogenesis.

This hypothesis is supported in this review by the fact that, among the studies presented in table 1, three articles [3,9,10] investigated both the L1 gene and the viral oncoproteins, with 54% of detection, considering the pre-chemotherapy rate by Islam et al., 2017[9]. On the other hand, those who sought to detect only L1 [8,10,22,24] or E6 and/or E7 [23], detected only 29.02% and 14.8% of viral genetic material, respectively.

In addition to the technique of using specific primers for E6/E7 showing good sensitivity and specificity [62], changes in the genetic material occur more frequently in the L1 gene than in E6 and E7, which can make it difficult to anneal the primer during PCR, which may lead to non-detection of the virus[60].

In contrast to what is expected, and the results obtained from the articles in table 1, there are studies that support the possibility of association without statistical significance (table 2) and studies that did not find evidence of this association (table 3). In Table 2, the lowest detection rate was among the studies that analyzed both the presence of L1 and E6/E7, with 23.05% of detection[13,35,36,38]. The study by Banerjee et al., 2015[25], which used specific primers for E2, E4, and L1, detected viral genetic material from HPV 6 and 16 in triple-negative breast cancer in 79% of cases.

A curious fact among the studies, which reported not having found evidence of an association between HPV and breast cancer (Table 3), is that in addition to the higher detection rate being among studies that only searched for L1 (average rate of 6,76%) [50,52,53], in the study by Aguayo et al., 2011[44], the viral genome was only detected when using primes for the L1 gene, with no detection with the use of primers for E6/E7.

Studies show that the presence of the viral genome is not a sufficient condition to establish a causal relationship[35]. Analysis of the association between the presence of HPV and the expression of oncoproteins E6 and E7 adjoins the tumor suppressor proteins p53, pRb, and p16ink4a in breast carcinomas, represent relevant findings, as they indicate that in addition to viral DNA, oncoproteins were identified in the tissues[64–66]. The possibility that E6 activity prevents p53 function in breast tissue has been associated

with aggressive clinical pathological behavior and poor prognosis in these patients[55,67,68].

The relationship between the presence of HPV DNA in non-cancerous tissue is still poorly understood [6,17,40], however, reports show the presence of hr-HPV in benign breast lesions of Australian and Turkish patients[23]. It is necessary to assess whether HPV infection in normal tissue becomes a favorable and/or necessary condition for the development of a malignant neoplasm [46].

It is noteworthy that the presence of HPV proteins per cell is sufficient for immortalization, cumulative changes [34,69], and neoplastic transformation, as occurs in SiHa cells, where there are 1-2 copies of HPV16 per cell [65]. Several studies have confirmed the presence of HPV DNA in breast tumors, however, the carcinogenic mechanisms are inconclusive [70]. On the other hand, in vivo, and in vitro functional studies confirmed the involvement of HPV infection in carcinogenesis, activation, progression, invasion, and metastasis in breast cancer[6].

3.6 INTEGRATION OF HPV INTO THE BREAST CANCER GENOME

As previously described, HPV may or may not integrate part of its genetic material into that of the host, presenting itself in an episomal, integrated, or mixed form. There is evidence that when the virus integrates, it loses the L1 gene, as well as E1 and E2[6,34,60,61], at the same time that it increases the expression of the viral proteins E6 and E7[3], responsible for the inactivation of the p53 and Rb genes, respectively.

Because viral integration, in addition to being associated with the inactivation of tumor suppressor genes, also acts on the activation of oncogenes, this type of presentation is associated with a greater relationship with neoplasms with a higher degree of malignancy[29,43,44]. Therefore, apart from just detecting or not the presence of HPV in the analyzed sample, it is important to assess its integration status [6]. Some studies in this review analyzed the association of viral status with the presence or absence of malignancy in the studied lesion, as well as with tumor characteristics [3,9,28,44].

The study by Khodabandehlou et al., 2019[3] showed the absence of E2 in 86% of the analyzed cases of malignant lesions, which supports the hypothesis that the integrated status of the virus would be related to carcinogenesis. Furthermore, an E2/E6 ratio <1 was found in 14% of malignant lesion cases, which suggests a mixed status of the virus (episomal + integrated). Isolated episomal status was not evidenced in any sample. These results are similar to those found by Islam et al in 2017[9], which showed integration of the viral genome in 87.5% of the malignant samples. In this study, 8.3% had mixed virus status and 4.2% had episomal status. In addition, the authors of this last study mentioned that they could not find statistical significance in relation to histopathological differences when related to viral status, probably because most of the samples presented with the integration of the genetic material.

Aguayo et al. 2011[44], in turn, showed viral integration in 100% of the 14 samples analyzed, as none showed amplification of the E2 gene. Wang et al., 2016[28], found, that in the 52 (35,6%) positive cases for HPV, in 146 breast cancer samples analyzed, the mixed status of the virus.

So, the detection of HPV with integrated or mixed status in this review, among the studies that did this analysis, ranged between 35.6%-100% in the samples diagnosed with cancer, while the episomal form ranged between 0-4.2 %, corroborating the hypothesis that the integrated form would be more associated with lesions with characteristics of malignancy.

IV. CONCLUSION

In this study, most of the articles analyzed showed a positive association between HPV and breast carcinoma, although some did not present a statistical “p-value”. The most common genotype was HPV 16, followed by HPV 18, high-risk genotypes that are also the most frequent among other types of carcinomas caused by this virus. The main histopathological diagnosis of HPV-related breast cancer was invasive ductal carcinoma, which, despite being the most frequent histological type among breast carcinomas, may also be related to the fact that the ducts are in contact with the external environment, increasing the chance of contamination. In addition, it is important to highlight the percentage difference between the detection of genetic material in the integrated form, found in 35.6%-100% of the cancer samples studied, and the episomal, found only between 0-4.2%, which is directly related to the expression of E6 and E7 proteins, which have their expression increased when the viral DNA integrates with that of the host, decreasing the activity of tumor suppressor genes and increasing the activity of oncogenes. The results of this study, in agreement with others included in our review, support the hypothesis that HPV may have a great influence on breast carcinogenesis.

REFERENCES

[1] Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

[2] MS - INCA. (2019). *Estimativa/2020 - Incidência de Câncer no Brasil*. http://www.inca.gov.br

[3] Khodabandehlou, N., Mostafaei, S., Etemadi, A., Ghasemi, A., Payandeh, M., Hadifar, S., Norooznezhad, A. H., Kazemnejad, A., & Moghoofei, M. (2019). Human papilloma virus and breast cancer : the role of inflammation and viral expressed proteins. *BMC Cancer*, *19*(61), 1–11. https://doi.org/101.1186/s12885-019-5286-0

[4] Lawson, J. S., Salmons, B., & Glenn, W. K. (2018). Oncogenic viruses and breast cancer: Mouse mammary tumor virus (MMTV), bovine leukemia virus (BLV), human papilloma virus (HPV), and Epstein-Barr virus (EBV). *Frontiers in Oncology*, *8*(JAN). https://doi.org/10.3389/fonc.2018.00001

[5] INCA. (2016). Estimativa 2016: Incidência de Câncer no Brasil. *Ministério Da Saúde - Instituto Nacional de Câncer José Alencar Gomes Da Silva*.

[6] G. de Lima, E., M.M. do Amaral, C., C.Q. Peixe, F., P.A.D. Gurgel, A., da Costa Silva Neto, J., & C. de Freitas, A. (2016). Putative Mechanisms of Viral Transmission and Molecular Dysregulation of Mammary Epithelial Cells by Human Papillomavirus: Implications for Breast Cancer. *Current Molecular Medicine*, *16*(7), 650–659. https://doi.org/10.2174/1566524016666160805120410

[7] El-Sheikh, N., Mousa, N. O., Tawfeik, A. M., Saleh, A. M., Elshikh, I., Deyab, M., Ragheb, F., Moneer, M. M., Kawashti, A., Osman, A., & Elrefaei, M. (2021). Assessment of Human Papillomavirus Infection and Risk Factors in Egyptian Women With Breast Cancer. *Breast Cancer: Basic and Clinical Research*, *15*, 1–10. https://doi.org/10.1177/1178223421996279

[8] Delgado-García, S., Martínez-Escoriza, J. C., Alba, A., Martín-Bayón, T. A., Ballester-Galiana, H., Peiró, G., Caballero, P., & Ponce-Lorenzo, J. (2017). Presence of human papillomavirus DNA in breast cancer: A Spanish case-control study. *BMC Cancer*, *17*(1), 1–11. https://doi.org/10.1186/s12885-017-3308-3

[9] Islam, S., Dasgupta, H., Roychowdhury, A., Bhattacharya, R., Mukherjee, N., Roy, A., Mandal, G. K., Alam, N., Biswas, J., Mandal, S., Roychoudhury, S., & Panda, C. K. (2017). Study of association and molecular analysis of human papillomavirus in breast cancer of Indian patients: Clinical and prognostic implication. *PLoS ONE*, *12*(2), 1–17. https://doi.org/10.1371/journal.pone.0172760

[10] Cavalcante, J. R., Pinheiro, L. G. P., Almeida, P. R. C. de, Ferreira, M. V. P., Cruz, G. A., Campelo, T. A., Silva, C. S., Lima, L. N. G. C., Oliveira, B. M. K. de, Lima, L. M., Feitosa, L. M. C., Pinheiro, A. C., & Frota, C. C. (2018). Association of breast cancer with human papillomavirus (HPV) infection in Northeast Brazil: molecular evidence. *Clinics (Sao Paulo, Brazil)*, *73*(13), e465. https://doi.org/10.6061/clinics/2018/e465

[11] Khodabandehlou, N., Mostafaei, S., Etemadi, A., Ghasemi, A., Payandeh, M., Hadifar, S., Norooznezhad, A. H., Kazemnejad, A., & Moghoofei, M. (2019). Human papilloma virus and breast cancer: The role of inflammation and viral expressed proteins. *BMC Cancer*, *19*(1), 1–11. https://doi.org/10.1186/s12885-019-5286-0

[12] Malhotra, G. K., Zhao, X., Band, H., & Band, V. (2010). Histological, molecular and functional subtypes of breast cancers. *Cancer Biology and Therapy*, *10*(10), 955–960. https://doi.org/10.4161/cbt.10.10.13879

[13] De Carolis, S., Storci, G., Ceccarelli, C., Savini, C., Gallucci, L., Sansone, P., Santini, D., Seracchioli, R., Taffurelli, M., Fabbri, F., Romani, F., Compagnone, G., Giuliani, C., Garagnani, P., Bonafè, M., & Cricca, M. (2019). HPV DNA Associates With Breast Cancer Malignancy and It Is Transferred to Breast Cancer Stromal Cells by Extracellular Vesicles. *Frontiers in Oncology*, *9*(September), 1–12. https://doi.org/10.3389/fonc.2019.00860

[14] Bernard, H. U., Burk, R. D., Chen, Z., van Doorslaer, K., Hausen, H. zur, & de Villiers, E. M. (2010). Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology*, *401*(1), 70–79. https://doi.org/10.1016/j.virol.2010.02.002

[15] Burk, R. D., Harari, A., & Chen, Z. (2013). Human papillomavirus genome variants. *Virology*, *445*(0), 232–243. https://doi.org/10.1016/j.virol.2013.07.018.Human

[16] Durzynska, J., Lesniewicz, K., & Poreba, E. (2017). Human papillomaviruses in epigenetic regulations. *Mutation Research - Reviews in Mutation Research*, *772*, 36–50. https://doi.org/10.1016/j.mrrev.2016.09.006

[17] Malhone, C., Longatto-Filho, A., & Filassi, J. R. (2018). Is Human Papilloma Virus Associated with Breast Cancer? A Review of the Molecular Evidence. *Acta Cytologica*, *62*(3), 166–177. https://doi.org/10.1159/000487700

[18] Band, V., Zajchowski, D., Kulesa, V., & Sager, R. (1990). Human papilloma virus DNAs immortalize normal human mammary epithelial cells and reduce their growth factor requirements. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(1), 463–467. https://doi.org/10.1073/pnas.87.1.463

[19] Li, J., Ding, J., & Zhai, K. (2015). Detection of human papillomavirus DNA in patients with breast tumor in China. *PLoS ONE*, *10*(8), 1–10. https://doi.org/10.1371/journal.pone.0136050

[20] Lonardo, A. Di, Venuti, A., & Marcante, M. L. (1992). Human papillomavirus in breast cancer. *Breast Cancer Research and Treatment*, *21*, 95–100.

[21] Sigaroodi, A., Nadji, S. A., Naghshvar, F., Nategh, R., Emami, H., & Velayati, A. A. (2012). Human papillomavirus is associated with breast cancer in the north part of Iran. *The Scientific World Journal*, *2012*, 1–8. https://doi.org/10.1100/2012/837191

[22] Piana, A. F., Sotgiu, G., Muroni, M. R., Cossu-rocca, P., Castiglia, P., & Miglio, M. R. De. (2014). *HPV infection and triple-negative breast cancers : an Italian case-control study*. *11*(1), 1–5. https://doi.org/10.1186/s12985-014-0190-3

[23] Fu, L., Wang, D., Shah, W., Wang, Y., Zhang, G., & He, J. (2015). Association of Human Papillomavirus Type 58 With Breast Cancer in Shaanxi Province of China. *Journal of Medical Virology*, *87*, 1034–1040. https://doi.org/10.1002/jmv.24142

[24] Afshar, R. M., Balar, N., Mollaei, H. R., Arabzadeh, S. A., & Iranpour, M. (2018). Low prevalence of human papilloma virus in patients with breast cancer, Kerman; Iran. *Asian Pacific Journal of Cancer Prevention*, *19*(11), 3039–3044.

https://doi.org/10.31557/APJCP.2018.19.11.3039

[25] Banerjee, S., Wei, Z., Tan, F., Peck, K. N., Shih, N., Feldman, M., Rebbeck, T. R., Alwine, J. C., & Robertson, E. S. (2015). Distinct microbiological signatures associated with triple negative breast cancer. *Scientific Reports*, *5*, 1–14. https://doi.org/10.1038/srep15162

[26] Yan, C., Teng, Z. P., Chen, Y. X., Shen, D. H., Li, J. T., & Zeng, Y. (2016). Viral etiology relationship between human papillomavirus and human breast cancer and target of gene therapy. *Biomedical and Environmental Sciences*, *29*(5), 331–339. https://doi.org/10.3967/bes2016.043

[27] Ilahi, N. E., Anwar, S., Noreen, M., Hashmi, S. N., & Murad, S. (2016). Detection of human papillomavirus-16 DNA in archived clinical samples of breast and lung cancer patients from North Pakistan. *Journal of Cancer Research and Clinical Oncology*, *142*(12), 2497–2502. https://doi.org/10.1007/s00432-016-2251-z

[28] Wang, D., Fu, L., Shah, W., Zhang, J., Yan, Y., Ge, X., He, J., Wang, Y., & Li, X. (2016). Presence of high risk HPV DNA but indolent transcription of E6/E7 oncogenes in invasive ductal carcinoma of breast. *Pathology Research and Practice*, *212*(12), 1151–1156. https://doi.org/10.1016/j.prp.2016.09.009

[29] Naushad, W., Surriya, O., & Sadia, H. (2017). Prevalence of EBV, HPV and MMTV in Pakistani breast cancer patients: A possible etiological role of viruses in breast cancer. *Infection, Genetics and Evolution*, *54*, 230–237. https://doi.org/10.1016/j.meegid.2017.07.010

[30] Salman, N. A., Davies, G., Majidy, F., Shakir, F., Akinrinade, H., Perumal, D., & Ashrafi, G. H. (2017). Association of High Risk Human Papillomavirus and Breast cancer : A UK based Study. *Nature Publishing Group*, 1–8. https://doi.org/10.1038/srep43591

[31] Wang, Y. X., Li, Y. Z., Zhang, Z. Y., Wang, J. Q., Cui, J., & Qian, X. L. (2017). HPV16 E6 Promotes Breast Cancer Proliferation via Upregulation of COX-2 Expression. *BioMed Research International*, *2017*, 1–12. https://doi.org/10.1155/2017/2948467

[32] Elamrani, A., Gheit, T., Benhessou, M., Mckay-chopin, S., Attaleb, M., Sahraoui, S., El, M., & Corbex, M. (2018). Prevalence of mucosal and cutaneous human papillomavirus in Moroccan breast cancer. *Papillomavirus Research*, *5*(December 2017), 150–155. https://doi.org/10.1016/j.pvr.2018.04.003

[33] Habyarimana, T., Attaleb, M., Mazarati, J. B., Bakri, Y., & El Mzibri, M. (2018). Detection of human papillomavirus DNA in tumors from Rwandese breast cancer patients. *Breast Cancer*, *25*(2), 127–133. https://doi.org/10.1007/s12282-018-0831-2

[34] Sher, G., Salman, N. A., Kulinski, M., Fadel, R. A., Gupta, V. K., Anand, A., Gehani, S., Abayazeed, S., Al-Yahri, O., Shahid, F., Alshaibani, S., Hassan, S., Zafar Chawdhery, M., Davies, G., Dermime, S., Uddin, S., Hossein Ashrafi, G., & Junejo, K. (2020). Prevalence and type distribution of high-risk human papillomavirus (HPV) in breast cancer: A Qatar based study. *Cancers*, *12*(6), 1–10. https://doi.org/10.3390/cancers12061528

[35] Herrera-Goepfert, R., Khan, N. A., Koriyama, C., Akiba, S., & Pérez-Sánchez, V. M. (2011). High-risk human papillomavirus in mammary gland carcinomas and non-neoplastic tissues of Mexican women: No evidence supporting a cause and effect relationship. *Breast*, *20*(2), 184–189. https://doi.org/10.1016/j.breast.2010.11.006

[36] Golrokh Mofrad, M., Sadigh, Z. A., Ainechi, S., & Faghihloo, E. (2021). Detection of human papillomavirus genotypes, herpes simplex, varicella zoster and cytomegalovirus in breast cancer patients. *Virology Journal*, *18*(1), 1–10. https://doi.org/10.1186/s12985-021-01498-z

[37] Glenn, W. K., Heng, B., Delprado, W., Iacopetta, B., Whitaker, N. J., & Lawson, J. S. (2012). Epstein-Barr Virus, Human Papillomavirus and Mouse Mammary Tumour Virus as Multiple Viruses in Breast Cancer. *PLoS ONE*, *7*(11). https://doi.org/10.1371/journal.pone.0048788

[38] Herrera-goepfert, R., Vela-chávez, T., Carrillo-garcía, A., Lizano-soberón, M., Amador-molina, A., Oñate-ocaña, L. F., & Hallmann, R. S. (2013). High-risk human papillomavirus (HPV) DNA sequences in metaplastic breast carcinomas of Mexican women. *BMC Cancer*, *13*(445), 1–8. https://doi.org/10.1186/1471-2407-13-445

[39] Pereira Suarez, A. L., Lorenzetti, M. A., Gonzalez Lucano, R., Cohen, M., Gass, H., Vazquez, P. M., Gonzalez, P., Preciado, M. V., & Chabay, P. (2013). Presence of Human Papilloma Virus in a Series of Breast Carcinoma from Argentina. *PLoS ONE*, *8*(4), 1–5. https://doi.org/10.1371/journal.pone.0061613

[40] Ahangar-Oskouee, M., Shahmahmoodi, S., Jalilvand, S., Mahmoodi, M., Ziaee, A. A., Esmaeili, H. A., Keshtvarz, M., Pishraft-Sabet, L., Yousefi, M., Mollaei-Kandelous, Y., Mokhtari-Azad, T., & Nategh, R. (2014). No detection of "High-risk" human papillomaviruses in a group of Iranian women with breast cancer. *Asian Pacific Journal of Cancer Prevention*, *15*(9), 4061–4065. https://doi.org/10.7314/APJCP.2014.15.9.4061

[41] Corbex, M., Bouzbid, S., Traverse-Glehen, A., Aouras, H., McKay-Chopin, S., Carreira, C., Lankar, A., Tommasino, M., & Gheit, T. (2014). Prevalence of Papillomaviruses, Polyomaviruses, and Herpesviruses in Triple-Negative and Inflammatory Breast Tumors from Algeria Compared with Other Types of Breast Cancer Tumors. *PLoS ONE*, *9*(12), 1–15. https://doi.org/10.1371/journal.pone.0114559

[42] Wang, T., Zeng, X., Li, W., Zhu, H., Wang, G., Liu, X., Lv, Y., Wu, J., Zhuang, X., Zhang, J., Zhao, Y., Huang, H., Fan, J., Yao, Q., He, C., Zhang, X., Huang, C., Chen, J., & Wang, L. (2014). Detection and analysis of human papillomavirus (HPV) DNA in breast cancer patients by an effective method of HPV capture. *PLoS ONE*, *9*(3), 3–9. https://doi.org/10.1371/journal.pone.0090343

[43] Ohba, K., Ichiyama, K., Yajima, M., Gemma, N., Nikaido, M., Wu, Q., Chong, P. P., Mori, S., Yamamoto, R., Wong, J. E. L., & Yamamoto, N. (2014). In vivo and in vitro studies suggest a possible involvement of HPV infection in the early stage of breast carcinogenesis via APOBEC3B induction. *PLoS ONE*, *9*(5), 1–12. https://doi.org/10.1371/journal.pone.0097787

[44] Aguayo, F., Khan, N., Koriyama, C., González, C., Ampuero, S., Padilla, O., Solís, L., Eizuru, Y., Corvalán, A., & Akiba,

S. (2011). Human papillomavirus and Epstein-Barr virus infections in breast cancer from chile. *Infectious Agents and Cancer*, *6*(1), 7. https://doi.org/10.1186/1750-9378-6-7

[45] Kouloura, A., Nicolaidou, E., Misitzis, I., Panotopoulou, E., Smyrniotis, V., Corso, G., Veroneci, P., & Arkadopoulos, N. (2018). HPV infection and breast cancer. Results of a microarray approach. *Breast*, *40*(2018), 165–169. https://doi.org/10.1016/j.breast.2018.05.010

[46] Chang, P., Wang, T., Yao, Q., Lv, Y., Zhang, J., Guo, W., Wang, L., & Chen, J. (2012). Absence of human papillomavirus in patients with breast cancer in north-west China. *Medical Oncology*, *29*(2), 521–525. https://doi.org/10.1007/s12032-011-9945-5

[47] Hedau, S., Kumar, U., Hussain, S., Shukla, S., Pande, S., Jain, N., Tyagi, A., Deshpande, T., Bhat, D., Mir, M. M., Chakraborty, S., Singh, Y. M., Kumar, R., Somasundaram, K., Bharti, A. C., & Das, B. C. (2011). Breast cancer and human papillomavirus infection: No evidence of HPV etiology of breast cancer in Indian women. *BMC Cancer*, *11*(1), 27. https://doi.org/10.1186/1471-2407-11-27

[48] Herrera-Romano, L., Fernández-Tamayo, N., Gómez-Conde, E., Reyes-Cardoso, J. M., Ortiz-Gutierrez, F., Ceballos, G., Valdivia, A., Piña, P., & Salcedo, M. (2012). Absence of human papillomavirus sequences in epithelial breast cancer in a Mexican female population. *Medical Oncology*, *29*(3), 1515–1517. https://doi.org/10.1007/s12032-011-0059-x

[49] Peng, J., Wang, T., Zhu, H., Guo, J., Li, K., Yao, Q., Lv, Y., Zhang, J., He, C., Chen, J., Wang, L., & Jin, Q. (2014). Multiplex PCR/mass spectrometry screening of biological carcinogenic agents in human mammary tumors. *Journal of Clinical Virology*, *61*(2), 255–259. https://doi.org/10.1016/j.jcv.2014.07.010

[50] Gannon, O. M., Antonsson, A., Milevskiy, M., Brown, M. A., Saunders, N. A., & Bennett, I. C. (2015). No association between HPV positive breast cancer and expression of human papilloma viral transcripts. *Scientific Reports*, *5*, 18081. https://doi.org/10.1038/srep18081

[51] Karimi, M., Khodabandehloo, M., Nikkhoo, B., & Ghaderi, E. (2016). No Significant Association between Human Papillomavirus and Breast Cancer, Sanandaj, Iran. *Asian Pacific Journal of Cancer Prevention*, *17*(10), 4741–4745. https://doi.org/10.22034/apjcp.2016.17.10.4741

[52] Bakhtiyrizadeh, S., Hosseini, S. Y., Yaghobi, R., Safaei, A., & Sarvari, J. (2017). Almost complete lack of human cytomegalovirus and human papillomaviruses genome in benign and malignant breast lesions in Shiraz, Southwest of Iran. *Asian Pacific Journal of Cancer Prevention*, *18*(12), 3319–3324. https://doi.org/10.22034/APJCP.2017.18.12.3319

[53] Ngamkham, J., Karalak, A., Chaiwerawattana, A., Thanasutthichai, S., Sukarayodhin, S., Boonmark, K., Phansri, T., & Laochan, N. (2017). Prevalence of Human Papillomavirus Infection in Breast Cancer Cells from Thai Women. *Asian Pacific Journal of Cancer Prevention*, *18*, 1839–1845. https://doi.org/10.22034/APJCP.2017.18.7.1839

[54] Li, Y., & Xu, C. (2017). Human Papillomavirus-Related Cancers. In *Infectious Agents Associated Cancers: Epidemiology and Molecular Biology. Advances in Experimental Medicine and Biology* (Vol. 1018, pp. 23–34). https://doi.org/10.1007/978-981-10-5765-6

[55] Wang, Y. W., Zhang, K., Zhao, S., Lv, Y., Zhu, J., Liu, H., Feng, J., Liang, W., Ma, R., & Wang, J. (2017). HPV Status and Its Correlation with BCL2, p21, p53, Rb, and Survivin Expression in Breast Cancer in a Chinese Population. *BioMed Research International*, *2017*, 1–7. https://doi.org/10.1155/2017/6315392

[56] Augustin, J. G., Lepine, C., Morini, A., Brunet, A., Veyer, D., Brochard, C., Mirghani, H., Péré, H., & Badoual, C. (2020). HPV Detection in Head and Neck Squamous Cell Carcinomas: What Is the Issue? *Frontiers in Oncology*, *10*(September), 1–13. https://doi.org/10.3389/fonc.2020.01751

[57] Demarco, M., Carter-Pokras, O., Hyun, N., Castle, P. E., He, X., Dallal, C. M., Chen, J., Gage, J. C., Befano, B., Fetterman, B., Lorey, T., Poitras, N., Raine-Bennett, T. R., Wentzensen, N., & Schiffman, M. (2018). Validation of a Human Papillomavirus (HPV) DNA cervical screening test that provides expanded HPV typing. *Journal of Clinical Microbiology*, *56*(5), 1–10. https://doi.org/10.1128/JCM.01910-17

[58] Eslamifar, A., Ramezani, A., Azadmanesh, K., Bidari-Zerehpoosh, F., Banifazl, M., & Aghakhani, A. (2015). Assessment of the association between human papillomavirus infection and Breast carcinoma. *Iranian Journal of Pathology*, *10*(1), 41–46. https://doi.org/10.7508/ijp.2015.01.006

[59] Tota, J. E., Struyf, F., Merikukka, M., Gonzalez, P., Kreimer, A. R., Bi, D., Castellsagué, X., De Carvalho, N. S., Garland, S. M., Harper, D. M., Karkada, N., Peters, K., Pope, W. A. J., Porras, C., Quint, W., Rodriguez, A. C., Schiffman, M., Schussler, J., Rachel Skinner, S., … Lehtinen, M. (2017). Evaluation of Type Replacement Following HPV16/18 Vaccination: Pooled Analysis of Two Randomized Trials. *Journal of the National Cancer Institute*, *109*(7), 1–9. https://doi.org/10.1093/jnci/djw300

[60] Dictor, M., & Warenholt, J. (2011). Single-tube multiplex PCR using type-specific E6/E7 primers and capillary electrophoresis genotypes 21 human papillomaviruses in neoplasia. *Infectious Agents and Cancer*, *6*(1), 1. https://doi.org/10.1186/1750-9378-6-1

[61] Pinatti, L. M., Walline, H. M., & Carey, T. E. (2018). Human Papillomavirus Genome Integration and Head and Neck Cancer. *Journal of Dental Research*, *97*(6), 691–700. https://doi.org/10.1177/0022034517744213

[62] Derbie, A., Mekonnen, D., Woldeamanuel, Y., Van Ostade, X., & Abebe, T. (2020). HPV E6/E7 mRNA test for the detection of high grade cervical intraepithelial neoplasia (CIN2+): A systematic review. *Infectious Agents and Cancer*, *15*(1), 1–10. https://doi.org/10.1186/s13027-020-0278-x

[63] Tjalma, W. A. A., & Depuydt, C. E. (2013). Cervical cancer screening: Which HPV test should be used - L1 or E6/E7? *European Journal of Obstetrics and Gynecology and Reproductive Biology*, *170*(1), 45–46. https://doi.org/10.1016/j.ejogrb.2013.06.027

[64] Den Boon, J. A., Pyeon, D., Wang, S. S., Horswill, M., Schiffman, M., Sherman, M., Zuna, R. E., Wang, Z., Hewitt,

S. M., Pearson, R., Schott, M., Chung, L., He, Q., Lambert, P., Walker, J., Newton, M. A., Wentzensen, N., & Ahlquist, P. (2015). Molecular transitions from papillomavirus infection to cervical precancer and cancer: Role of stromal estrogen receptor signaling. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(25), E3255–E3264. https://doi.org/10.1073/pnas.1509322112

[65] Pal, A., & Kundu, R. (2020). Human Papillomavirus E6 and E7: The Cervical Cancer Hallmarks and Targets for Therapy. *Frontiers in Microbiology*, *10*(January). https://doi.org/10.3389/fmicb.2019.03116

[66] Ngan, C., Lawson, J. S., Clay, R., Delprado, W., Whitaker, N. J., & Glenn, W. K. (2015). Early human Papilloma virus (HPV) Oncogenic influences in breast cancer. *Breast Cancer: Basic and Clinical Research*, *2015*(9), 93–97. https://doi.org/10.4137/BCBCR.S35692

[67] Westrich, J. A., Warren, C. J., & Pyeon, D. (2017). Evasion of host immune defenses by human papillomavirus. *Virus Research*, *231*, 21–33. https://doi.org/10.1016/j.virusres.2016.11.023

[68] James, C. D., Morgan, I. M., & Bristol, M. L. (2020). The relationship between estrogen-related signaling and human papillomavirus positive cancers. *Pathogens*, *9*(5), 1–16. https://doi.org/10.3390/pathogens9050403

[69] Santos, J. M. O., da Silva, S. P., Costa, N. R., Gil da Costa, R. M., & Medeiros, R. (2018). The role of microRNAs in the metastatic process of high-risk HPV-induced cancers. *Cancers*, *10*(12), 1–15. https://doi.org/10.3390/cancers10120493

[70] Islam, M. S., Chakraborty, B., & Panda, C. K. (2020). Human papilloma virus (HPV) profiles in breast cancer: future management. *Annals of Translational Medicine*, *8*(10), 650–650. https://doi.org/10.21037/atm-19-2756