Human papillomavirus in breast cancer of patients with cervical intraepithelial neoplasia or cervical cancer history. A systematic review and meta-analysis

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Summary

Purpose: Many cohort studies and meta-analyses support the oncogenic role of the human papilloma virus (HPV) on breast tissue. However, only a few studies examine the association between HPV-positive breast cancer and the prior history of high grade cervical intraepithelial neoplasia (CIN) or cervical cancer. The present systematic review and meta-analysis aimed to determine whether women with a history of high grade CIN or cervical cancer are at a higher risk of developing HPV-positive breast cancer.

Methods: MEDLINE, CENTRAL and Scopus databases as well as "gray literature" sources were searched for case-control studies, detecting and genotyping HPV genome in breast cancer patients with and without a history of CIN or cervical cancer, from inception to October 23, 2020.

Results: The meta-analysis included three case-control studies with 265 breast cancer patients in total. HPV related breast cancer was associated with a history of high grade CIN or cervical cancer [pooled odds ratio (OR) = 7.98, 95% confidence interval (CI), 1.84 to 34.67]. This association remained regarding HPV-16 related breast cancer (pooled OR = 7.60, 95% CI, 1.75 to 33.00).

Conclusions: HPV was detected more frequently in breast cancer patients with CIN or cervical cancer history. Therefore, further research is necessary to understand better the HPV transmission route to the breast.

Key words: breast cancer, cervical cancer, cervical intraepithelial neoplasia (CIN), human papillomavirus, HPV

Introduction

Breast cancer is the most common malignancy in women worldwide. According to the latest data from the World Health Organization (WHO), in 2018, there were 2,088,849 new breast cancer cases and 626,679 deaths due to breast cancer worldwide [1]. Risk factors for breast cancer include increased exposure to endogenous and exogenous estrogens, nulliparity, increased alcohol consumption, familial predisposition, and mutations in the BRCA1 and BRCA2 genes [2]. Various viruses such as mouse mammary tumor virus (MMTV), Epstein-Barr virus (EBV), and human papillomavirus (HPV) have also been implicated in breast cancer pathogenesis [3]. Many cohort and case-control studies have detected the HPV genome in breast cancer tissue at a rate of 1.6-86.2% [4-7]. A recent meta-analysis consisting of 37 case-control studies showed that HPV is more common in cancer lesions than in benign lesions or...
normal mammary gland (Standardized Odds Ratio: 6.22) [8]. In the same meta-analysis, HPV types 16, 18 and 53 were significantly associated with breast cancer.

HPV is a double-stranded circular DNA virus that replicates in the nucleus of mucosal cells and is responsible for 99.7% of cervical intraepithelial neoplasias (CIN) and invasive cervical cancer [9]. 70% of cases are due to types 16 and 18, while for the remaining pre-cancer cervical lesions and cervical cancers, the high-risk HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 are responsible [10]. In recent years, vaccination of young girls against the HPV virus with vaccines covering HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58 have shown increased primary prevention against cervical cancer [11,12].

Recent studies support the possible development of secondary HPV-related cancers in sites outside the genital system in patients treated for high-grade CIN (II-III) or cervical cancer [13–15]. Preti et al in Italy performed an analysis of data from 5184 women surgically treated for CIN (II-III) and found 173 second primary cancers in sites such as the anus, vagina, vulva, oropharynx, lungs and bladder. An increased risk of second primary occurrence was also found for breast and ovarian cancer [16]. Another study describes possible ways in which HPV can reach the breast tissue from the genital area and lead to carcinogenesis. In particular, three modes of possible transmission are proposed. The first is that of hematogeneous or lymphogeneous transmission. The second is during sexual intercourse through the nipple or injuries to the skin covering the breast due to genital-breast sexual activity. Finally, a third possible transmission mode is from the oral cavity due to oral sexual activity [17].

This systematic review and meta-analysis aimed to highlight whether women with prior cervical neoplasia or cervical cancer carry an increased risk of HPV-positive breast cancer compared to women without cervical neoplasia or cervical cancer.

### Methods

This meta-analysis was designed in compliance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) [18] statement and was prospectively registered in the PROSPERO database (registration number CRD42020214084).

#### Literature search

MEDLINE (via PubMed), Scopus, and Cochrane Library (CENTRAL) databases as well as “grey literature” sources [repositories, catalogues (EThOS), websites (OpenGrey, GetNet International) and conference proceedings] were searched for eligible studies, from inception to October 23, 2020. Basic terms used in search strings were “human papillomavirus,” “HPV,” “breast,” in both free text and Medical Subject Headings (MeSH) format. Finally, the published systematic reviews and the bibliographies of the included studies were screened for additional studies not previously identified.

#### Study eligibility

A study was eligible for this meta-analysis only if it was in compliance with the predetermined inclusion and exclusion criteria. Case-control studies assessing the association of prior cervical intraepithelial neoplasia or cervical cancer with HPV-positive breast cancer were eligible for inclusion. Studies should have two arms: breast cancer patients with prior cervical neoplasia or cervical cancer (cases) and breast cancer patients without any history of cervical neoplasia or cervical cancer (controls). Cohorts, case reports and case series were excluded from this meta-analysis. The proportion of detected HPV-positive breast cancer should have been described in both arms. No restrictions were set about either the type of HPV or breast cancer. As a second primary cancer diagnosis, breast cancer should have been histologically verified, regardless of stage. High-grade CIN (II-III) or invasive cervical cancer should have been diagnosed before or no later than 18 months after a breast cancer diagnosis in all cases. Women in the control group should have been diagnosed with breast cancer and have normal cervical cytology within five years of their breast cancer diagnosis.
cancer diagnosis. Studies were eligible regardless of the type of tissue examined (fresh frozen tissue or paraffin-embedded tissue). Human papillomavirus detection and genotyping with PCR-based methods were essential for the eligibility of the study. No country or publication date restrictions were applied. Exclusion criteria were simple cervical HPV infection without cervical intraepithelial neoplasia, and control group consisted of normal breast tissue.

Data extraction

Two investigators (EM, AP) independently extracted data after reviewing the selected studies. Any discrepancy was discussed with all investigators and resolved in a consensus meeting. Furthermore, we contacted the corresponding authors when further information was needed. Two e-mails were sent to them with a 14-day difference. If no response was received, the study was excluded. Abstracted items included: i) study characteristics (first author's name and year of publication, country or region, sample size, age of women, type of breast cancer, type of HPV detected); ii) design of the selected studies (inclusion/exclusion criteria, HPV detection method, breast cancer detection method); iii) proportions of HPV-positive breast cancer cases both in patients with and without prior cervical neoplasia or cervical cancer.

Quality assessment

The methodological quality of the included studies was assessed using the Newcastle-Ottawa Scale and evaluated independently by two researchers (EM and CC) to ascertain the risk of bias. Any disagreement was resolved by consensus with a third researcher (KD).

Data synthesis and analysis

HPV-positive and HPV-negative breast cancers were tabulated in women with and without prior cervical neoplasia or cervical cancer, stratified by study. These numbers were used to calculate pooled odds ratio (OR) and 95% confidence intervals (CI) using the Mantel-Haenszel statistical method. Moreover, we collected the available statistics of HPV 16 subtype. The random-effects model was applied to pool the data. The presence of between-study heterogeneity was measured with the I² index, considering values of 25%, 50%, and 75% as low, moderate, and high, respectively. R language (packages "meta", "metafor" and "dmetar") was used for the statistical analyses.

Results

Search results and study characteristics

The search strategy yielded 1935 studies. After removing the duplicates and reviewing the titles and abstracts, 161 remained for full-text review. Out of them, 158 were excluded, leaving three case-control studies for the quantitative analysis. It is noteworthy that one case-control study was exclud-

| Reference Number | First author | Year of publication | Country | Age (years) | History | Time between diagnosis and histological confirmation (years) | Tissue type | Detection method | HPV 16 Subtype | HPV 18 Subtype | HPV 51 Subtype | HPV 56 Subtype | HPV 52 Subtype | HPV 58 Subtype |
|------------------|-------------|---------------------|--------|-------------|---------|-------------------------------------------------|------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| [31]             | Widenschneider | 2004               | Austria | 50.1        | Cervical cancer | 1.81 | PET | PCR-EIA | 7/11 | 1/11 | 6/11 |
| [33]             | Hennig       | 1999               | Norway  | 49.6        | CIN III | 5.58 | PET | PCR-ISH | 19 | 19/41 | 0/9 |

Data are given as mean. CIN: Cervical Intraepithelial Neoplasia; FFPET: formalin-fixed paraffin-embedded tissue; PET: paraffin-embedded tissue; SPF: strip-based reverse hybridization; PCR-DEIA-LIPA: strip-based reverse hybridization-PCR-deoxyribonucleic acid-ligase enzyme immunoassay.
ed because the control arm consisted of normal breast tissue samples [19]. Another case-control study was excluded because the case group consisted of breast cancer patients with a prior HPV infection and not a history of CIN or cervical cancer [20]. A study from Hansen et al was excluded because the outcome was the standardized incidence ratio of malignant breast tumors in women with squamous or glandular precancer, without detecting HPV in these women [21]. Finally, an Iranian study from Hossein et al was not eligible for the quantitative analysis because of the determination of the HPV prevalence in cervix lesions and breast cancer tissues in different women and not in breast cancer patients with prior history of CIN or cervical cancer [22]. The flow diagram of the study selection process is shown in Figure 1.

The three included case-control studies were published between 1999 and 2018, from Austria, Denmark, and Norway. The largest sample size included 193 patients, and the smallest 22 patients. A total of 265 patients were enrolled in this study. One study included women with a history of CIN III, one with a history of CIN III or worse, and one with cervical cancer. There was no statistically significant difference in mean age between women

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**Table 2. Quality assessment of the studies. Each asterisk shows if individual criterion was fulfilled**

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Figure 2. Association between HPV-related breast cancer and CIN/cervical cancer history.

Figure 3. Pooled analysis for HPV-16.
in the two arms of the studies. Tissue type was formalin fixed-paraffin embedded for one study and paraffin-embedded for the other two studies. HPV detection and genotyping were performed using PCR-ISH in one study, PCR-EIA in the second study, and SPF PCR-DEIA-LIPA in the third study. Characteristics of the included studies and baseline characteristics of the women are summarized in Table 1.

Association between HPV-related breast cancer and CIN/cervical cancer history

In all studies, 28 breast cancer patients out of the 145 with a history of CIN or cervical cancer were found HPV-positive, while only two breast cancer patients out of the 120 without a history of high grade CIN or cervical cancer were HPV-positive. The pooled odds ratio (OR) was 7.98 [95% confidence interval (CI) 1.84 to 34.67], which provided evidence that HPV-related breast cancer is increased among patients with prior history of high grade CIN or cervical cancer. The results of the pooled analysis for the included studies are shown in Figure 2.

Out of the HPV-related breast cancer patients with a prior history of high grade CIN or cervical cancer, HPV type 16 was detected in 26 (92.9%). Examining the association of only HPV-16 related breast cancer with a history of high grade CIN or cervical cancer, the pooled OR was 7.60 (95% CI 1.75 to 33.00). The results of the pooled analysis for HPV-16 are shown in Figure 3.

Quality assessment

All of the three included studies underwent methodological quality assessment according to the Newcastle-Ottawa Quality Assessment Scale for case-control studies [25]. A detailed presentation of the systematic error risk assessment of all included studies is given in Table 2.

Discussion

A comprehensive systematic review was conducted, addressing worldwide HPV prevalence rates among breast cancer patients with a history of high grade CIN or cervical cancer. As far as we are aware, this is the first systematic review and meta-analysis on this topic. Three case-control studies were included, consisting of 265 patients in total. The present study showed that HPV was detected 8-fold more frequently in breast cancer patients with a history of high grade CIN or cervical cancer. Besides, pooled analysis for high-risk HPV types showed that the frequency of HPV detection was 7.6-fold higher in breast cancer patients with a history of high grade CIN or cervical cancer.

Numerous studies in the literature have described the possible oncogenic role of HPV genome on breast tissue [24]. HPV genome affects epithelial cells and immortalizes them. Furthermore, it was mentioned that in HPV-positive breast cancer tissues, there was a decreased expression of p53 and p21 proteins [25-28]. However, it is not yet determined the possible route of HPV transmission from primary cancer areas, such as the anogenital area, to the breast [29,30]. Windischwendter et al conducted HPV detection and genotyping in breast cancer tissues and in lymph nodes and serum samples to elucidate a possible hematogenic spread of the HPV virus. According to the results of the study, the hematogenic spread might be a possible transmission route [31]. Although it is challenging to determine the exact time of the viral transmission, it is more likely to occur before or at the time of clinical diagnosis of second primary cancer. Furthermore, another mode of transmission might be during sexual intercourse through the nipple or injuries to the skin covering the breast due to genital-breast sexual activity or via the oral cavity due to oral intercourse [17].

A key strength of the present study lies within the fact that this is the first systematic review and meta-analysis in literature, trying to elucidate an association between prior history of high grade CIN or cervical cancer and HPV-positive breast cancer. Furthermore, it was designed in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA), and the included studies were assessed using the Newcastle-Ottawa Scale.

Limitations of the present study are the small number of the included studies and the enrolled patients. Furthermore, studies have differences in their design, PCR techniques, and genotyping techniques. It would be beneficial when all studies contain detection and genotyping of HPV in regional lymph nodes and serum samples to extract more precise results about the virus’s transmission mode. Moreover, the use of paraffin-embedded breast tissues is associated with lower sensitivity in the detection of HPV genome, in particular, due to possible DNA degradation and cross-linking, in comparison with fresh frozen tissue samples or cytological specimens.

In conclusion, HPV was detected more frequently in breast cancer patients with a history of high grade CIN or cervical cancer. This finding's clinical implication is that HPV vaccination...
may contribute to primary prevention not only of cervical cancer but of breast cancer as well. However, it would be essential to conduct further well-designed case-control studies with larger sample size, leading to more reliable findings. Future studies could also focus on the association of hormone receptor status and tumor size with HPV prevalence in breast cancer.

**References**


**Author contributions**


**Conflict of interests**

The authors declare no conflict of interests.


