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Evaluating the Association between Human Papillomavirus and Vulvar Cancer: A Comprehensive Analysis Using Bradford Hill Criteria

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Abstract: *Background:* The role of human papillomavirus (HPV) in the development of vulvar cancer (VC) has been widely studied, but findings have been inconsistent. Despite numerous meta-analyses exploring the potential link between HPV and VC, the association remains controversial due to inherent limitations in meta-analytic methods. *Objectives:* To address this controversy, the study aims to investigate the potential link between HPV and VC using the Bradford Hill criteria, which offer a more comprehensive framework for establishing causation. *Methodology:* The study began by extracting all relevant studies on the association between HPV and VC from the PubMed database. The potential links were then assessed by examining the data using the major postulates of the Bradford Hill criteria. To ensure the reliability of the findings, the methodologies of the identified studies were critically evaluated to account for possible false-negative and false-positive results. *Results:* The assessment of previous studies against the Bradford Hill criteria revealed that the major postulates were not fulfilled. *Conclusion:* Based on the findings, it can concluded that there is no causal association between HPV and VC.

Keywords: Vulvar cancer (VC); Bradford Hill criteria; Human papillomavirus (HPV)

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1. Introduction

Vulvar cancer (VC) is basically a female genital tract disease, accounting for around 5% of all gynecological malignancies [1]. In the United States of America (USA), the VC prevalence has risen by 0.6% annually in the last decade [2]. The major and primary option for VC treatment is surgery. However, in selected cases, chemotherapy/radiotherapy is also used as an alternative treatment option [3]. VC is mainly divided into two distinct subtypes, having differential etiology: (1) Human papillomaviruses (HPVs) infection associated, and (2) HPV infection-independent VC [4].

Considering the participation of HPV in VC, different studies worldwide have documented the role of HPV in VC so far, but their results were contradictory [1,2,5]. Various groups of researchers used statistical meta-analysis to resolve this disagreement and obtain a more accurate association between HPV and VC. However, due to significant limitations of the statistical meta-analysis, including the inability to critically evaluate the methodologies, providing no information regarding the heterogeneity of the studied populations and publication biases, the evaluation of a correlation between HPV and VC is due to an additional strategy.

The study evaluated the correlation between HPV and VC using Bradford Hill criteria postulates. These postulates are worldwide effective for linking a presumed cause with an effect [6]. In the evaluation, the data of previous studies were analyzed to document whether or not previous studies met the Bradford Hill criteria postulates to declare a causal association between HPV and VC. To make the outcomes more authentic, the study also critically reviewed the methodologies of identified studies to address the propensity of false results.

2. Material and methods

The study implemented a two-phase methodology (**Figure 1**).

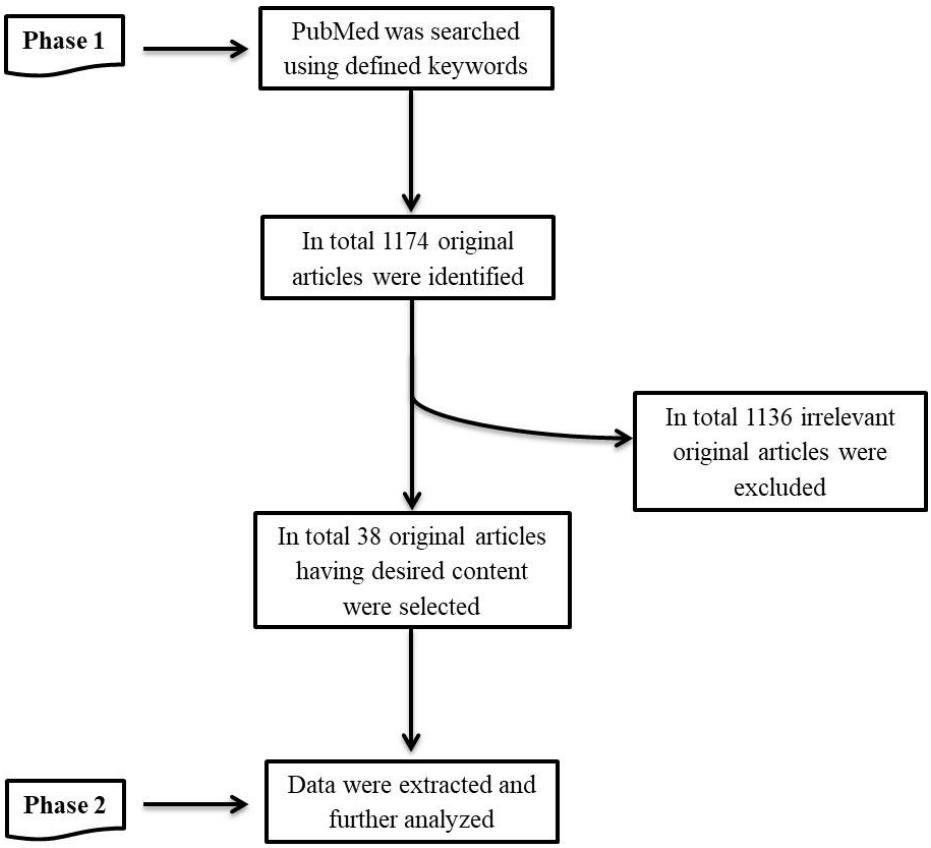

Figure 1. Overview of the methodology implemented during the present study.

2.1. Literature search

Related studies associating HPV with VC were searched via PubMed using the keywords “Vulvar Cancer” and “Human papillomavirus.” Additionally, “Retroviridae” and “Vulvar intraepithelial neoplasia” were also used as medical subject headings (MeSH) terms. All the original articles available until December 2020 were searched. In the end, a total of 1176 original articles were found.

2.2. Relevant data acquisition

Out of 1176 studies, a total of 38 relevant studies were shortlisted, which studied the association between HPV and VC, after reading their titles, abstracts and the complete article. In addition, a detailed table was built after acquiring the required data from shortlisted studies.

2.3. Evaluation of the results using the postulates of Bradford Hill criteria

Based on the acquired data, the selected studies were critically evaluated using eight major Bradford Hill criteria postulates:

(1) Strength,
(2) Temporality,
(3) Consistency,
(4) Plausibility,
(5) Biological gradient,
(6) Experiment,
(7) Specificity,
(8) Analogy.

The postulate's evaluation was descriptive, with no quantitative assigned score. The evidence for each postulate is given in **Table 1**, and the results part with a final verdict of whether or not the postulate was fulfilled.

3. Results

On PubMed, A total of 38 original studies [7–43] (**Table 1**) were identified worldwide that examined the potential link of HPV with VC. **Table 1** summarizes the selected studies and includes the important acquired data from these studies essential for the assessment of Bradford Hill criteria postulates including information of the studied population, names of the technique utilized for the HPV identification, targeted gene name, name of the HPV detected strain, CI and P values, name of the prevalent identified HPV strain, total analyzed samples count (normal, benign and VC) with respective population-wide detection positivity ratios.

The positivity ratio of HPV detection in the VC samples varied population-wide from 3.3% [12] to 76.5% [35]. In normal and adjacent or benign samples, it varied from 40% [30] to 97.1% [14].

Table 1. Summary of the detection of HPV and positivity rate in normal and vulvar cancer samples relative to the different selected articles.

Studied population	Technique used for viral genome detection	Target gene/ protein	Prevalent strain	Number of the normal sample screened	Percentage positivity of HPV in normal samples (%)	Number of the adjacent or benign samples screened	Percentage positivity of HPV in adjacent or benign samples (%)	Number of the total vulvar cancer samples screened	Percentage positivity of HPV in vulvar cancer samples (%)	References	*P*-value	CI (%)
Spain	PCR	L1	16	0	0	0	0	98	19.4	[7]	-	-
	PCR	L1	2, 29	0	0	0	0	902	3.3	[12]	-	-
	PCR	-	16	0	0	0	0	94	19	[13]	-	-
	PCR	L1	16, 18	0	0	0	0	37	30.3	[32]	-	-
	PCR	L1	16	0	0	0	0	92	14.7	[36]	-	-
Russia	PCR	-	-	0	0	0	0	58	20.6	[9]	-	-
Brazil	Immunohisto-chemistry	-	16, 18	0	0	0	0	55	7.3	[11]	-	-
	PCR	-	16	0	0	0	0	85	45	[24]	-	-
United states	PCR	L1	16	0	0	68	97.1	176	68.8	[14]	< 0.0001	-
	PCR	L1	16	0	0	0	0	56	27	[25]	-	-
	PCR	L1	16	0	0	0	0	17	76.5	[35]	-	-
	PCR	L1	16	0	0	0	0	116	69.8	[35]	-	-
	PCR	L1, E6, E7	16	0	0	0	0	55	60	[29]	-	-
	PCR	-	-	0	0	0	0	39	59	[16]	-	-
Germany	PCR	L1	16	0	0	0	0	183	43.7	[33]	-	-
	PCR	-	-	0	0	0	0	36	50	[15]	-	-
China	PCR	-	16, 18	0	0	0	0	8	75	[16]	-	-
Canada	PCR	-	-	0	0	0	0	193	40	[19]	-	-
	Immunohisto-chemistry	P16	-	0	0	0	0	197	40	[27]		
	PCR	L1	16	0	0	0	0	43	51	[43]	-	-
Korea	Hybrid Capture 2 test	-	16, 18	0	0	0	0	45	42.86	[20]	-	-
Poland	Linear Array HPV Genotyping test	-	16	0	0	0	0	46	15	[21]	-	-

Table 1 (Continued)

Studied population	Technique used for viral genome detection	Target gene/ protein	Prevalent strain	Number of the normal sample screened	Percentage positivity of HPV in normal samples (%)	Number of the adjacent or benign samples screened	Percentage positivity of HPV in adjacent or benign samples (%)	Number of the total vulvar cancer samples screened	Percentage positivity of HPV in vulvar cancer samples (%)	References	*P*-value	CI (%)
	SPF10-LiPA25 system	L1	-	0	0	0	0	85	45	[39]	-	-
Japan	PCR	E7	16, 18	0	0	0	0	31	12.8	[22]	-	-
Sweden	PCR	-	16	0	0	0	0	130	30.8	[23]	-	-
	PCR	L1	16, 18	0	0	0	0	75	31	[26]	-	-
	Immunohisto-chemistry	-	-	0	0	0	0	217	22	[21]	-	-
Israel	PCR	-	16, 18	0	0	0	0	14	64.2	[28]	-	-
Thailand	PCR	L1	16	0	0	20	40	34	47.1	[30]	-	-
	PCR	L1	16	0	0	0	0	47	62	[37]	-	-
Australia	PCR	L1	16	0	0	0	0	521	38.6	[34]	-	-
Greece	PCR	E6, E7	16	0	0	6	71.4	6	50	[40]	-	-
Netherlands	PCR	L1	16	0	0	0	0	130	34.6	[41]	-	-
	PCR	L1	-	0	0	0	0	60	32	[8]	-	-
	PCR	L1	16	0	0	0	0	66	18	[18]	-	-
United Kingdom	PCR	-	16	0	0	112	87	62	52	[42]	-	-
Switzerland	*In situ* hybridization	-	16	0	0	0	0	44	9	[10]	-	-
Austria	PCR	L1	16	0	0	0	0	41	23	[31]	-	-

3.1. The evidence for each of the Bradford-Hill postulates

3.1.1. Strength

The existence of a weak association does not rule out the possibility of a causal association. However, this situation is more likely to be clarified by undetected prejudices. The point that stronger relationships tend to be more causative is rational. In total, 4 case-control studies were found in the literature reporting an association between HPV and VC [14,30,40,42]. None of the case-control studies have reported the CI, and only Gargano JW *et al.* (2012) have reported the *P*-value, lower HPV detection ratio in VC samples as compared to controls in the United States population [14]. However, none of the studies reported both CI and *P*-value. These data overall support a negligible strength of association between HPV and VC.

3.1.2. Consistency

Among some of the case-control studies, Ngamkham J *et al.* (2016) have reported higher HPV detection ratios in VC samples relative to controls, while three studies Gargano JW *et al.* (2012), Tsimplaki E *et al.* (2009) and Wakeham K *et al.* (2017) have documented the opposite results [14,30,40,42]. Therefore, consistent findings have not been observed in different populations strengthening the existence of an actual effect.

3.1.3. Biological gradient

In certain circumstances, the effect can be the outcome of the minor existence of a factor. In other cases, generally,, greater exposures lead to the higher induction of an effect. Viral load measurements may predict whether HPV differential viral load leads to differential outcomes in VC. Unfortunately, no study has reported the HPV viral load either in VC samples or controls. Therefore, the biological gradient postulate was not fulfilled.

3.1.4. Temporality

Temporality refers to the necessity for HPV to precede VC. The HPV detection ratios scenario in the current study has shown different outcomes. Out of 4 cross-sectional studies, Gargano JW *et al.* (2012), Tsimplaki E *et al.* (2009) and Wakeham K *et al.* (2017) have reported higher HPV detection ratio in normal controls relative to VC samples [14,40,42]. Moreover, in all case-control studies, HPV was detected in both normal and VC samples. Thus, such conflicting results failed to fulfill the temporal postulates.

3.1.5. Plausibility

Plausibility refers to a proper mechanism between cause and effect. HPV is well recognized as a potent inhibitor of TP53 in cervical cancer by making aE6/E6AP/p53 complex, resulting in the degradation of TP53 protein [44]. In the literature, 6 studies were found by analyzing the association between HPV presence and expression variations in TP53 level, they failed to validate their results [11,16,18,22,28,29]. Thus, the role of HPV in the etiology of VC is biologically not plausible.

3.1.6. Experiment

This postulate refers to the evidence from either animal or clinical studies. Evidence based on animal models and clinical studies, however, were absent in all the studies found in literature. Therefore, this postulate was not fulfilled.

3.1.7. Specificity

Causation is possible if a certain population develops VC in a certain region where the suspected cause is not clarified otherwise. The higher the specificity of the association between a factor and its effect, the more precise the relationship between a factor and its effect. VC is a multi-factorial disease and together with HPV the role of other non-infectious factors and oncogenic viruses (Epstein–Barr virus and Human Herpesvirus) in the development of VC is also well-studied worldwide [45,46]. Thus, the complexity of the involved factors in VC development suggested no specificity.

3.1.8. Analogy

The similar diseases to VC that can be considered to be VC analogous are breast and cervical cancer caused by other viral agents like Epstein–Barr virus (EBV) and Mouse mammary tumor virus (MMTV) [47,48]. However, the role of MMTV and EBV in the development of breast and cervical cancer is not yet fully established. Thus, in the present study, the scenario of analogy also suggests no association between HPV and VC.

4. Discussion

VC is a rare women malignancy that affects millions of people all over the world [49]. So far, many studies have been conducted worldwide documenting the relationship between HPV and VC to identify the possible oncogenic pathways regulating HPV in the development of VC. However, the findings were inconsistent. In addition, different groups of scientists worldwide have also performed a statistical meta-analysis to generate a more meaningful relationship between HPV and VC; due to statistical meta-analysis shortcomings, scientists yet again failed to find a reliable relationship between HPV and VC. Therefore, this study aims to find a relationship between HPV and VC using Bradford Hill criteria postulates.

In total 38 original articles were included in the present study [7-43]. The HPV detection ratio reported in these studies was varied between 3.3% [12] to 76.5% [35] in VC samples. Ngamkham J *et al.* (2016) reported that the positivity ratio of HPV detection was higher in the VC samples relative to controls [30]. However, Gargano JW *et al.* (2012), Tsimplaki E *et al.* (2009) and Wakeham K *et al.* (2017) reported a greater HPV detection ratio in the controls relative to the VC samples [14,40,42].

To our knowledge, no study has applied the Bradford Hill postulates so far to identify the association between HPV and VC. However, Awadh A *et al.* (2017) utilized these postulates to analyze the causal association between Zika infection and microcephaly, and they suggested no link between the studied parameters [50].

Since the initial identification of HPV in VC, more evidence has become available. The study systematically applied Bradford Hill's postulates on the available evidence to find an association between HPV and VC. The results were not in favor of a casual association. Therefore, this study speculated that HPV, along with other different viruses like human immunodeficiency virus (HIV) and hepatitis and C virus (HCV and B), as well as other genetic abnormalities, smoking, and alcohol consumption, increases the risk of developing VC by affecting the body's immune system [51].

Moreover, deficiencies, as well as some of the major drawbacks linked with the methodologies of the included studies, have been discussed below.

4.1. Possible causes of false-negative

Few studies did not detect HPV in any of the VC or control samples they were utilizing. How can we be sure

that the negative results were not because of the low-quality DNA? Several studies used positive control to address the question [8,12,14,17,21,24,26,29,30,34-38,40-43,47]. However, seven studies did not utilize the positive control in their experiment, so there is no way to validate their negative findings [9,13,15,22,31-33]. Primer selection targeting L1 and E1 genes of HPV might be inefficient for detecting HPV presence in advanced carcinoma and thus results in a false negative since L1 and E1 regions might be lost during viral genome integration with the genome of the host, whereas the E6/E7 regions remained consistently present in any circumstances so, this is the plausible explanation for the lower HPV detection ratios in different studies [10,12].

4.2. Possible causes of the false-positive

Most of the summarized studies utilized Polymerase Chain Reaction (PCR) for the detection of HPV and none have used any second technique to validate their PCR results [7-9,12-19,22-26,28-38,40-44]. In HPV-positive VC patients, expression profiling of various genes such as *p14*, *p16*, *p53*, *RB*, and others may be used as a surrogate biomarker. In addition to HPV detection, expression profiling of these biomarkers was also done by a few studies to validate their findings further, and all the studies failed to validate their findings with respect to surrogate biomarkers [7,11,16,18,21-25,28,31,39,41]. These inconsistencies in the previous studies' results pose a significant question mark as to the choice of suitable methods and their sensitivities.

4.3. Comparison of normal, benign and malignant samples

Case-control studies are essential when looking for a causal association between the cause and the disease. Few of the selected studies analyzed the VC samples only, which did not allow a comparison with normal, adjacent or benign and VC samples [7-13,15-29,31-39,41,43]. However, on the other side, few of the selected studies analyzed both normal or adjacent/benign and VC samples, and this comparison revealed a higher HPV detection ratio in VC samples in Ngamkham J *et al.* (2016)'s study, while lower in studies done by Gargano JW *et al.* (2012), Tsimplaki E *et al.* (2009) and Wakeham K *et al.* (2017) in comparison to the control [14,30,40,42]. However, no study has found a correlation between HPV and a certain VC subtype or histologic grade.

5. Conclusion

The findings of this study indicate no causal association between HPV and VC. However, due to methodological constraints in previous studies, further experiments are recommended to establish a definitive role of HPV in the etiology of VC.

Disclosure statement

The authors declare no conflicts of interest.

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