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LACK OF CORRELATION BETWEEN HUMAN PAPILLOMAVIRUS AND NASOPHARYNGEAL CANCER: A COMPREHENSIVE ASSESSMENT THROUGH SYSTEMATIC META-ANALYSIS

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Abstract

Objectives: Decoding the involvement of human papillomavirus (HPV) in nasopharyngeal cancer (NPC) has yielded contradictory findings worldwide. Despite various statistical meta-analyses exploring this association, controversy persists due to inherent limitations of meta-analysis. In this study, we aimed to investigate the potential link between HPV and NPC using an alternative method, the Bradford Hill criteria, to provide a clearer perspective.

Methods: PubMed was utilized to extract studies associating HPV with NPC. We evaluated the potential association using the Bradford Hill criteria postulates, scrutinizing available data on HPV in NPC and normal/benign samples. Rigorous assessment of study methodologies enhanced the authenticity of our findings, considering the possibility of false-negative and false-positive results.

Results: A comprehensive evaluation against Bradford Hill criteria revealed unfulfilled major postulates, including strength, temporality, consistency, plausibility, biological gradient, experiment, specificity, and analogy.

Conclusion: Our findings suggest no causal association between HPV and NPC.

Keywords: Nasopharyngeal cancer (NPC); Bradford Hill criteria; Human papillomavirus (HPV)

Introduction

Nasopharyngeal carcinoma (NPC) constitutes a mere 0.6% of global cancer cases, with prevalence rates varying significantly across different populations [1-3]. Notably, China reports an estimated prevalence of approximately 30 cases per 100,000, while North America has less than 1 case per 100,000 [4, 5]. In the United Kingdom (UK), NPC is uncommon, with a prevalence rate estimated at 0.3-0.4 cases per 100,000 [6].

Historically, the carcinogenic role of Epstein-Barr virus (EBV) in nasopharyngeal cancer has been well-established. However, an increasing body of evidence suggests a potential association between human papillomavirus (HPV) and NPC [7, 8].

Given the involvement of HPV in NPC, numerous global studies have explored its role in the disease. However, the findings from these studies, as documented in various publications [9-22], have been inconsistent. In an attempt to reconcile these discrepancies and establish a more precise association between HPV and NPC, several research groups have employed statistical meta-analysis. Despite this approach, the method has significant limitations, such as its inability to critically assess methodologies, lack of information regarding population heterogeneity in the studies, and susceptibility to publication bias. Therefore, an additional strategy is required to comprehensively evaluate the correlation between HPV and NPC.

In our investigation, we assessed the association between HPV and NPC by applying the Bradford Hill criteria postulates. These criteria are globally recognized for establishing a connection between a presumed cause and an effect [20]. During the evaluation, we scrutinized the data from prior studies to determine whether they fulfilled the Bradford Hill criteria postulates, indicating a causal link between HPV and NPC. Furthermore, to enhance the credibility of our findings, we conducted a thorough review of the methodologies employed in the identified studies to mitigate the potential for inaccurate results.

Methods

In assembling the current systematic meta-analysis, we adhered rigorously to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Checklist, along with the Meta-analysis of Observational Studies in Epidemiology guidelines (10). An outline of the methodology employed in our study is presented in Fig. 1.

Literature identification

We conducted a search for relevant studies linking HPV with NPC on PubMed, utilizing the keywords "Nasopharyngeal cancer" AND "Human papillomavirus." Additionally, "Retroviridae" AND "Nasopharyngeal neoplasm" were employed as Medical Subject Headings (MeSH) terms. Our search encompassed all original articles available up to December 2022.

Studies selection

During the initial phase, three authors identified pertinent studies by reviewing titles and abstracts. Subsequently, in the second step, the full texts of the identified relevant studies underwent further screening.

Eligibility criteria and relevant data acquisition

Out of a total of 343 studies, only 35 relevant studies were selected, focusing on the association between HPV and NPC. Furthermore, a comprehensive table was constructed, incorporating essential data obtained from these selected studies. This included information on (1) the studied population, (2) the techniques employed for HPV identification, (3) the targeted gene name and the HPV strain detected, (4) Confidence Intervals (CI) and P values, (5) the name of the prevalent identified HPV strain, and (6) the total count of analyzed samples (normal, benign, and EC) along with their respective population-wide detection positivity ratios.

Quality assessment

The chosen studies underwent quality assessment using the Newcastle-Ottawa scale tool (11). This tool meticulously evaluated various parameters, including the sampling plan, description of statistical analysis, and the reported outcomes.

Evaluation of the results using the postulates of Bradford Hill criteria

Using the obtained data, we conducted a thorough assessment of the chosen studies based on the eight key Bradford Hill criteria postulates: (1) Strength, (2) Temporality, (3) Consistency, (4) Plausibility, (5) Biological gradient, (6) Experiment, (7) Specificity, and (8) Analogy (12).

The assessment of the postulates was descriptive, without assigning a quantitative score. The evidence for each postulate is presented in Table 1 and the results section, concluding with a final determination of whether the postulate was satisfied or not.

Results

A total of 35 original studies [9-43] investigating the potential association of HPV with NPC were identified globally through PubMed. Table 1 provides a summary of these selected studies, presenting crucial data essential for assessing the Bradford Hill criteria postulates. This includes details about the studied population, the techniques used for HPV identification, the targeted gene name, the detected HPV strain name, Confidence Intervals (CI) and P values, the name of the prevalent identified HPV strain, and the total count of analyzed samples (normal, benign, and NPC) with their respective population-wide detection positivity ratios.

The positivity ratio of HPV detection in the NPC samples was varied population-wide from 0% [20, 32] to 84.3% [24]. While the positivity ratio of HPV detection in normal samples was varied from 0% [37] to 5.6% [36].

Evidence for each of the Bradford-Hill postulates

Strength

The presence of a weak association does not necessarily exclude the possibility of a causal link; however, in such instances, the likelihood of clarification may be impeded by undetected biases. The notion that stronger relationships are more likely to be causative is reasonable. A total of four case-control studies [15, 31, 36, 37] from the literature were identified, reporting on the association between HPV and NPC. None of these studies provided P-values and Confidence Intervals (CI), with one study [37] indicating a higher HPV detection ratio in NPC samples compared to controls. All the studies, conducted in China and Germany, reported a significant association between HPV and NPC. Overall, these findings suggest a minimal strength of association between HPV and NPC.

Consistency

Among the case-control studies [15, 31, 36, 37], one study [37] reported a higher HPV detection ratio in NPC samples compared to controls, while another study [36] documented the opposite results. Consequently, consistent findings have not been observed across different populations, further reinforcing the notion of the existence of a real effect.

Biological gradient

Under specific conditions, the effect may result from the minimal presence of a factor, while in other instances, a higher exposure typically leads to a more pronounced induction of the effect. The assessment of viral load measurements could potentially predict whether differential HPV viral loads contribute to varied outcomes in NPC. However, none of the identified studies reported HPV viral load in either NPC samples or control samples. As a result, the biological gradient postulate was not satisfied.

Temporality

Temporality involves the necessity for HPV to precede NPC. The outcomes of HPV detection ratios in the present study presented varied scenarios. Specifically, two cross-sectional studies [20, 32] reported no HPV detection in NPC samples, while no study indicated the absence of HPV detection in both NPC and control samples. One study [36] reported a higher HPV detection ratio in normal controls compared to NPC samples. Additionally, in some case-control studies [15, 31, 36], HPV was detected in both normal and NPC samples, showcasing conflicting results that failed to fulfill the temporal postulates. Another study [37] reported HPV detection only in NPC samples and not in control samples.

Plausibility

Plausibility refers to the existence of a plausible mechanism between the cause and effect. In cervical cancer, HPV is well-established as a potent inhibitor of TP53 through the formation of an E6/E6AP/p53 complex, leading to the degradation of TP53 protein [44]. In the literature, four studies [10, 12, 43] investigated the association between HPV presence and variations in TP53 expression. They validated their findings by confirming that TP53 was up-regulated in some cases [12, 43] and down-regulated in others [10] among NPC patients. Consequently, the biological implausibility of HPV playing a role in the etiology of NPC is evident.

Experiment

This postulate pertains to evidence derived from either animal or clinical studies. Unfortunately, none of the studies identified in the literature provided evidence based on animal models or clinical studies. Consequently, this postulate remains unfulfilled.

Specificity

Causation becomes plausible when a particular population develops NPC in a specific region, but the suspected cause remains unclear. The precision of the relationship between a factor and its effect increases with higher specificity of the association. NPC is acknowledged as a multi-factorial disease [45], and the global studies on NPC development extensively consider the roles of not only HPV but also other non-infectious factors and oncogenic viruses (such as EBV and John Cunningham virus) [46, 47]. Consequently, the complexity of the factors involved in NPC development suggests a lack of specificity.

Analogy

Diseases similar to NPC, which can be considered analogous, include breast cancer and cervical cancer caused by other viral agents such as Epstein–Barr virus (EBV) and Mouse mammary tumor virus (MMTV) [48, 49]. However, the roles of MMTV and EBV in the development of breast cancer and cervical cancer are not fully established. Consequently, the analogy scenario in the present study also suggests no discernible association between HPV and NPC.

Discussion

Nasopharyngeal carcinoma (NPC) constitutes only 0.6% of all global human cancers, with incidence rates showing significant variation across different populations [1]. Numerous studies worldwide have explored the association between HPV and NPC, aiming to identify potential oncogenic pathways influenced by HPV in NPC development. However, these studies have yielded inconsistent findings [50-53]. Attempts to establish a more conclusive relationship through statistical meta-analyses by various research groups globally have been hindered by the limitations of this approach, leading to a lack of a reliable association between HPV and NPC. Therefore, the present study aims to ascertain the relationship between HPV and NPC using Bradford Hill criteria postulates.

To the best of our knowledge, no study has employed the Bradford Hill postulates to investigate the association between HPV and NPC. However, it is noteworthy that a study utilized these postulates to analyze the causal relationship between Zika infection and microcephaly. Their findings suggested no link between the parameters under study [54].

Since the initial identification of HPV in NPC, a growing body of evidence has emerged. We systematically applied Bradford Hill's postulates to the available evidence to discern any association between HPV and NPC. However, the results did not support a causal association. Consequently, we hypothesize that factors such as HPV, along with other viruses like human immunodeficiency virus (HIV) and hepatitis C virus (HCV and B), as well as genetic abnormalities, smoking, and alcohol consumption, collectively contribute to an increased risk of developing NPC by impacting the body's immune system [55]. Additionally, deficiencies and major drawbacks associated with the methodologies of the included studies have been discussed below.

Possible causes of the false-positive results

The majority of the summarized studies relied solely on PCR [9-22] for HPV detection, while a subset of 17 studies [23-36, 38, 40, 43] incorporated a second technique to validate their PCR results. The additional techniques employed for validation included In-situ hybridization (ISH) [23-28, 30-33, 35, 36, 38], immunohistochemistry (IHC) [23, 25-28, 34, 43], dot hybridization [40], with results often deviating from the PCR outcomes. Notably, one study [33] demonstrated concordant results with PCR. Certain studies [29, 37, 41] exclusively utilized IHC, while others [39, 42] performed only ISH for HPV detection.

The expression profiling of various genes, including p14, p16, p53, RB, and others, has been explored as a surrogate biomarker in HPV-positive NPC patients. Many studies [10-12, 22-27, 29-38, 41, 43] not only conducted HPV detection but also carried out expression profiling of these biomarkers to validate their findings. Among them, 21 studies [10, 12, 22-27, 29-35, 37, 38, 41, 43] successfully validated their results by analyzing p53, RB, and other surrogate biomarkers, while the remaining 3 studies [11, 36] failed to validate their findings concerning surrogate biomarkers. These inconsistencies in previous study results raise significant questions regarding the selection of appropriate methods and their sensitivity.

Possible causes of false-negative results

The reliability of negative results in studies that did not detect HPV in NPC or control samples raises concerns about the potential influence of low-quality DNA. Several studies addressed this issue by using positive controls [9-12, 14, 20-23, 25, 28, 32, 35, 36]. However, other studies [13, 15-19, 24, 26, 27, 29-31, 33, 34, 37-43] did not incorporate positive controls in their experiments, leaving their negative findings without validation.

The choice of primers targeting L1 and E1 genes of HPV may be inefficient for detecting HPV presence in advanced carcinoma, potentially leading to false-negative results. This is because the L1 and E1 regions may be lost during viral genome integration with the host genome, while the E6/E7 regions consistently remain present. Therefore, the absence of positive findings in certain studies [9-11, 14, 15, 22, 23, 26, 30, 31, 36, 38] could be plausibly explained by the selection of primers that are not sensitive to integrated viral genomes.

Comparison of normal, benign, and malignant samples

Case-control studies are crucial when investigating a potential causal association between a factor and a disease. Some of the selected studies focused solely on NPC samples [9-14, 16-30, 32-35, 38-43], limiting the ability to compare with normal or adjacent/benign samples. Conversely, other studies [15, 31, 36, 37] analyzed both normal or adjacent/benign and NPC samples. In [37], this comparison revealed a higher HPV detection ratio in NPC samples compared to controls, while [36] showed a higher HPV prevalence in control samples than in NPC samples. However, two studies

[15, 31] did not perform a comparison between control and NPC cases. Notably, no study identified a correlation between HPV and a specific NPC subtype or histologic grade.

Conclusion

The findings of the current study did not establish a causal relationship between HPV and NPC. Nevertheless, recognizing the limitations in the methodologies employed by previous studies to detect HPV in NPC, it is advisable to conduct additional experiments for conclusive evidence regarding the potential role of HPV in NPC etiology.

Ethics approval and consent to participate

NA

Availability of data and material

NA

Competing interests

None to declare

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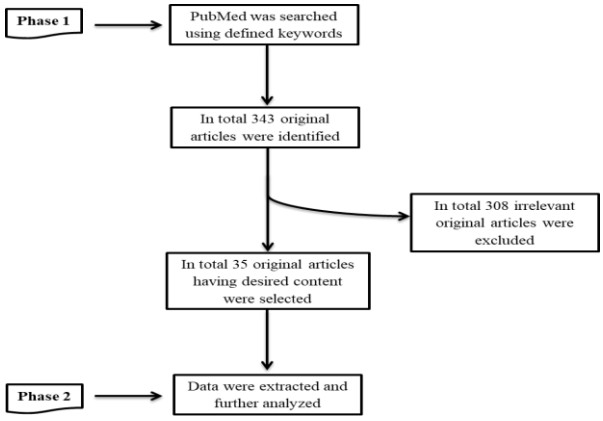

Fig. 1: Overview of the methodology used in the present study.

Table 1: Information of the HPV positivity ratios in controls and OC samples retrieved from the selected articles.

Studied Population	Technique used for viral genome detection	Target gene/protein	Prevalent strain	Number of normal samples screened(control)	Percentage positivity of HPV in normal samples (%)	Number of the adjacent or benign samples screen	Percentage positivity of HPV in adjacent or benign samples (%)	Number of the total nasopharyngeal cancer samples screened	Percentage positivity of HPV in nasopharyngeal cancer samples (%)	References	p-value	CI
USA	PCR, IHC, ISH	L1	16,18,31,33,35,4551,52,56,58,59,66,68,73					61	29.5	[23]		
	PCR, ISH	E6	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 73					89	84.3	[24]		
	ISH, PCR, IHC	E6, E7	16,18					30	E6-46.4 E7-53.6	[25]	0.37	
	PCR.	L1	16					30	23	[9]		
	PCR.	L1, E1	16					17	52.9	[10]		
	PCR, ISH, IHC	L1	6, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 73					18	50	[26]		
	ISH, IHC	E6/7	16,18					125	10	[27]		
	IHC, ISH							14	50	[28]		
	IHC							90	10	[29]		
UK	ISH, PCR	L1	16,18,31,33,35,39,45,1,52, 6, 58 ,56					67	16.4	[30]		
Morocco	PCR.	L1	16, 18, 31, 33, 35, 45 and 59.					70	34	[11]		
Germany	ISH, ICH, PCR	E6, E7, L1	16, 18, 31, 33, 35, 45, 52 and 58	142	Positivity was observed but not calculated			98	18	[31]		
China& USA (Collaboration)	PCR, ISH, IHC	E7	16					86 108	0 4.6	[32]		
Japan	PCR, IHC, ISH	E7	16,18,31,33,35,39,45,52,56,58,59,68					59	3.4	[33]		
	PCR.	E6 E7	16, 18, 31, 33, 35, 52, or 58					25	12	[12]	0.035	0.05-0.9
	ISH, IHC		16,18					956	32	[13]		
	IHC, PCR.	E6, e7	6, 18, 31, 33, 35, 52 and 58					10	30	[34]		
Finland	PCR, IHC, ISH	E6/7	16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73,					150	14	[35]	0.12	0.16-1.17
India	PCR.	L1						20	5	[14]		
Taiwan	PCR, ISH	L1	6, 11, 16, 18, 26, 31, 32, 33, 35, 37, 39, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 72, 74, 82	40	35			43	31	[36]		
China	PCR.	L1, E6	16,18	25	Positivity was observed but not calculated			70	2.9	[15]		
	PCR.	E6	16,18,45					88	51	[16]		
	IHC	E6	16	12	0			56	35	[37]		
Ghana	PCR.	E6/E7	16, 18, 31, 33, 35, 44, 42, 43, 45, 56, 52, and 58,					72	19.23	[21]		
South Africa	PCR.	L1, e6	16,18,31,45,3,58					3	33.33	[22]		
Czech Republic	PCR, ISH	L1, e7/6	18					62	1.6	[38]		
Mexico	PCR.		18,13,31,16,35					16	81.3	[17]		
Greece	PCR.	E7						63	14	[18]		
Iran	ISH		6/11, 16/18					20	20	[39]		
Iran	PCR.		6,11,16,18					41	22	[19]		
Denmark	PCR, dot hybridization		6, 11, 16, and 18					23	17	[40]		
Turkey	IHC							82	1.2	[41]		
	ISH		(16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 66					56	3.6	[42]		
Hongkong	PCR.	E6	16,18					16	0	[20]		
South Korea	PCR, IHC							46	6.5	[43]		

PCR = Polymerase chain reaction, ISH = In-situ hybridization,
IHC = Immunohistochemistry