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HEAD AND NECK CANCER RISK AND HUMAN PAPILLOMAVIRUS: BRADFORD HILL CRITERIA BASED EVALUATION

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Abstract
The involvement of human papillomavirus (HPV) has effectively been decoded in Head and neck cancer (HNC) worldwide with contradicting findings. Although the different groups of researchers explored the potential association of HPV with HNC using statistical meta-analysis, however, the association remained still controversial due to the major shortcomings of meta-analysis. Therefore, we arranged the present study to investigate a potential link of HPV with HNC using an additional method (Bradford Hill criteria) which helps to get a more clear picture. Methodology: Initially using PubMed, we extracted all of the studies that associated HPV to HNC. Then, to assess the potential association of HPV with HNC, an examination of the available data on HPV in HNC, normal/benign samples was done using all the major Bradford Hill criteria postulates. Furthermore, to improve the authenticity of our findings, we have also critically evaluated the methodologies of the identified studies to check the possibility of false-negative and false-positive results. Results: After a careful assessment of the previous studies against Bradford Hill criteria postulates, we observed that all the major postulates were not fulfilled. Conclusion: Therefore, our findings recommended no casual association of HPV with HNC.

Key words: Head and neck cancer (HNC); Bradford Hill criteria; Human papillomavirus (HPV)

INTRODUCTION

Head and neck cancers include a diverse variety of tumors that have been clustered together as an entity, based on etiology, anatomy, and sensitivity to chemotherapy (1-3). These malignancies include tumors of the oral cavity, paranasal sinuses, nasal cavity, pharynx, and larynx that arise from the upper aerodigestive tract (4-6). Around 95% of head and neck cancer (HNC) comes from squamous cells and thus are referred to as head and neck squamous cell carcinoma (HNSCC) (7-9).
Alcohol consumption and tobacco smoking are classified as the major predisposing factors for developing HNC, but now Human Papillomavirus (HPV), particularly HPV-16, has also been identified to be linked with HNC (7, 10-13).

Keeping in view the role of HPV in the cancer in HNC, various studies have been conducted so far to document the role of HPV in the pathogenesis of HNC but their results were conflicting (14-22). Various group of researchers used statistical meta-analysis to resolve this disagreement and obtain more accurate association between HPV and HNC. However, due to significant limitations of the statistical meta-analysis including inability to critically evaluate the methodologies, providing no information regarding heterogeneity of the studied populations, and publication biasness, the evaluation of a correlation among HPV and HNC is due with an additional strategy.
In our study, we evaluated the correlation among HPV and HNC using Bradford Hill criteria postulates. These postulates are worldwide effective for linking a presumed cause with an effect [20]. In the evaluation, we analyzed the data of previous studies to document, whether or not previous studies met the Bradford Hill criteria postulates to declare a causal association among HPV and HNC. Additionally, to make our outcomes more authentic, we also critically reviewed the methodologies of identified studies to address the propensity of false results.

MATERIAL AND METHODS

In our study, we implemented a two-phase methodology (Fig. 1).

LITERATURE IDENTIFICATION

Related studies associating HPV with HNC were searched via PubMed using the keywords: “Head and neck cancer” AND “Human papillomavirus”. We also defined “Retroviridae” AND “Head and neck neoplasm” as medical subject headings (MeSH) terms. All the original articles were searched available till December 2020. In the end, we found a total of 6831 original articles.

RELEVANT DATA ACQUISITION

From 6831 original articles, in total 52 relevant studies were shortlisted which studied the association between HPV and HNC reading their titles, abstract, and the complete article. In addition, a detailed table was built after acquiring the required data from shortlisted studies..

EVALUATION OF THE RESULTS USING BRADFORD HILL CRITERIA POSTULATES

Based on the acquired data, we critically evaluated the selected studies using eight major Bradford Hill criteria postulates:
(1) Strength, **(2)** Temporality, **(3)** Consistency, **(4)** Plausibility, **(5)** Biological gradient, **(6)** Experiment, **(7)** Specificity, and **(8)** Analogy (23)**.**
The postulate’s evaluation was descriptive, with no quantitative assigned score. The evidence for each postulate is given in (Table 1) and results part with a final verdict of whether or not the postulate was fulfilled.

RESULTS

On PubMed, A total of 52 original studies (14-17, 24-71) (Table 1) were identified worldwide that examined the potential link of HPV with HNC. 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 HNC) with respective population-wide detection positivity ratios.
The positivity ratio of HPV detection in the HNC samples was varied population-wide from 3.33% (14) to 78% (15). While, the positivity ratio of HPV detection in normal and adjacent/benign samples was varied from 0% (33, 52, 55, 57, 64) to 55 (71) and 0% (65) to 82% (17), respectively.

THE EVIDENCE FOR EACH OF THE BRADFORD-HILL POSTULATES

STRENGTH

The existence of a weak association does not rule out the possibility of a causal association; however, weak 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, 17 case-control studies (16, 17, 24, 30, 33, 34, 36, 48, 52, 55-57, 63-65, 67, 71) were found in the literature reporting association between HPV and HNC. Only 05 (16, 24, 34, 56, 71) of them have reported the CI (16, 24, 34), P-values (56, 71), and higher HPV detection ratio in HNC samples as compare to controls except one study (17), and found a significant association between HPV and HNC in Mexican, Pakistani, Japanese and Chinese populations. However, none of the study reported both CI and P-value. These data overall support a negligible strength of association between HPV and HNC.

CONSISTENCY

Among 17 case-control studies, 15 studies (16, 24, 30, 33, 34, 36, 48, 52, 55-57, 63-65, 71) have reported the higher HPV detection ratios in HNC samples relative to controls while two studies (17, 67) have documented the opposite results. Therefore, consistent findings have not been observed in different populations using different populations strengthening the existence of a non-casual association.

BIOLOGICAL GRADIENT

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

TEMPORALITY

Temporality refers to the necessity for HPV to precede HNC. The HPV detection ratios scenario in the current study has shown different outcomes. In total, 11 case-control studies (16, 17, 24, 30, 34, 36, 48, 56, 63, 67, 71) reported that HPV was detected in both normal and HNC samples. Moreover, in two case-control studies (17, 67) HPV detection ratio was higher in normal controls relative to HNC sample. Such conflicting result thus, failed to fulfill the temporal postulates.

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 (72). In literature, five studies (35, 43, 57, 68, 72) were found analyzing the association between HPV presence and expression variations in TP53 level: they failed to validate their results. Thus, the role of HPV in the etiology of HNC is biologically not plausible.

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.

SPECIFICITY

Causation is possible if a certain population develop HNC in a certain region where the suspected cause is not clarified otherwise. Higher the specificity of the association between a factor and its effect, the more precise the relationship between a factor and its effect. HNC is multi-factorial disease (73) and together with HPV the role of other non-infectious factors and oncogenic viruses (Epstein–Barr virus and John Cunningham virus) in the development of HNC is also well studied worldwide (74-76). Thus, the complexity of the involved factors in HNC development suggested no specificity.

ANALOGY

The similar diseases to HNC that can considered to be HNC analogous are breast cancer and cervical cancer caused by other viral agents like Epstein–Barr virus (EBV), and Mouse mammary tumor virus (MMTV) (77, 78). However, the role of MMTV and EBV in the development of breast cancer and cervical cancer is yet not fully established. Thus, in the present study, the scenario of analogy also suggests no association between HPV and HNC.

DISCUSSION

HNC is the sixth most common types of cancer that affect that affect people all over the world. So far, many studies were conducted worldwide documenting the relationship between HPV and HNC to identify the possible oncogenic pathways regulating by HPV in the development of HNC, however the findings were inconsistent. In addition, a statistical meta-analysis has also been performed by different groups of scientist worldwide to generate a more meaningful relationship between HPV and HNC, due to statistical meta-analysis shortcoming, scientists yet again failed to find a reliable relationship among HPV and HNC. Therefore, in the present study our aim is to find a relationship between HPV and HNC using Bradford Hill criteria postulates.

In total 52 original articles (14-17, 24-71) were included in the present study. The HPV detection ratio reported in these studies was varied between 3.33% (14) to 78% (15) in HNC samples. In most of the case-control studies (16, 24, 30, 33, 34, 36, 48, 52, 55-57, 63-65, 71) the positivity ratio of HPV detection was higher in the HNC samples as compared to the controls while in two studies (17, 67) HPV positivity ratio was higher in the controls as compared to the HNC samples.

Best to our knowledge, no study has applied the Bradford Hill postulates so far to identify the association between HPV and HNC, However, one study utilized these postulates to analyze the causal association between Zika infection and microcephaly, and they suggested no link between the studied parameters (79).

Since, from the initial identification of HPV in HNC, more evidence has become available. We systematically applied Bradford Hill's postulates on the available evidence to find an association between HPV and HNC. The results were not in favor of a casual association. Thus, it was proposed that HPV might combine with the other viruses such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV), and other factors including genetic abnormalities, smoking, alcohol consumption to increase a person's risk of developing HNC by affecting the body's immune system (80).

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

POSSIBLE CAUSES OF FALSE-NEGATIVE RESULTS

Some studies failed to detect HPV presence in any of the cancerous or normal controls they were investigating. How we can be sure that negative results for HPV detection were not due to the poor quality of the extracted DNA? Many studies utilized positive control to avoid such situations (14, 15, 17, 24, 25, 28-30, 33, 34, 37, 38, 40, 44, 46, 47, 50, 62, 63, 65, 66, 69, 71) but few studies (26, 41, 42, 48, 52-54, 56) did not use positive control so there is no way to confirm their negative results. Primers selection targeting L1 and E1 genes of HPV might be inefficient for detecting HPV presence in the advanced carcinoma and thus results in false negative, since L1 and E1 regions

might be lost during viral genome integration with the genome of host, whereas, the E6/E7 regions remained consistently present in any circumstances so, this is the plausible explanation for the negative results of (52, 55, 57, 64, 65) studies.

POSSIBLE CAUSES OF THE FALSE-POSITIVE RESULTS

Most of the studies that we summarized used PCR technique (14-17, 24-30, 33-38, 40-42, 44, 46-54, 56-58, 60, 62-66, 68, 69, 71) for the detection of HPV and none of them utilized second technique to confirm their positive results of PCR, except n = 5 studies (29, 33, 40, 58, 64) which utilized Immunohistochemistry (33, 40, 58), Hybrid capture 2 test (29) and Southern blotting technique (64) and the results of their second techniques have deviated from the first one. In HPV positive HNC patients, expression profiling of various genes such as p14, p16, p53, RB, and others may be used as a surrogate biomarker. Along with HPV detection, these surrogate biomarkers were also analyzed by some studies (27, 28, 33, 35, 43, 44, 46, 49, 54, 57, 58, 68) to further validate their findings, out of which two studies (28, 46) has validated their findings by analyzing p16 as surrogate biomarker while the other studies (27, 33, 35, 43, 44, 49, 54, 57, 58, 68) were failed to validate their findings with surrogate biomarkers. Such deviations in the results of previous studies raise a big question mark on the selection of appropriate technique and their sensitivities.

COMPARISON OF NORMAL, BENIGN AND MALIGNANT SAMPLES

Case-control studies are necessary to establish a causal relationship between the causative agent and the disease. Some of the studies we summarized analyzed only the HNC samples (14, 15, 25-29, 31, 32, 35, 37-47, 49-51, 53, 54, 58-62, 66, 68-70) and did not allow us to compare their results with normal or adjacent/benign controls. However, most of the studies (16, 17, 24, 30, 33, 34, 36, 48, 52, 55-57, 63-65, 67, 71) also analyzed the normal and adjacent/benign tissues along with HNC samples and comparison of their results demonstrated that HPV detection positivity ratios in HNC samples were higher in (16, 24, 30, 33, 34, 36, 48, 52, 55-57, 63-65, 71) studies while lower in two studies (17, 67) as compared to the normal controls. However, no study has found a correlation between HPV and a certain HNC subtype or histologic grade.

CONCLUSION

The results of the present study failed to prove a causal relationship between HPV and HNC. However, due to the limitations of the methodologies used by the previous studies to detect the presence of HPV in HNC, additional experiments are recommended to prove the HPV etiology in HNC.

LIST OF ABBREVIATION

HPV = Human Papillomavirus
HNC = Head and neck cancer
HIV = Human immunodeficiency virus
HCV = Hepatitis C virus
PCR = Polymerase chain reaction

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CONFLICT OF INTEREST

None to declare

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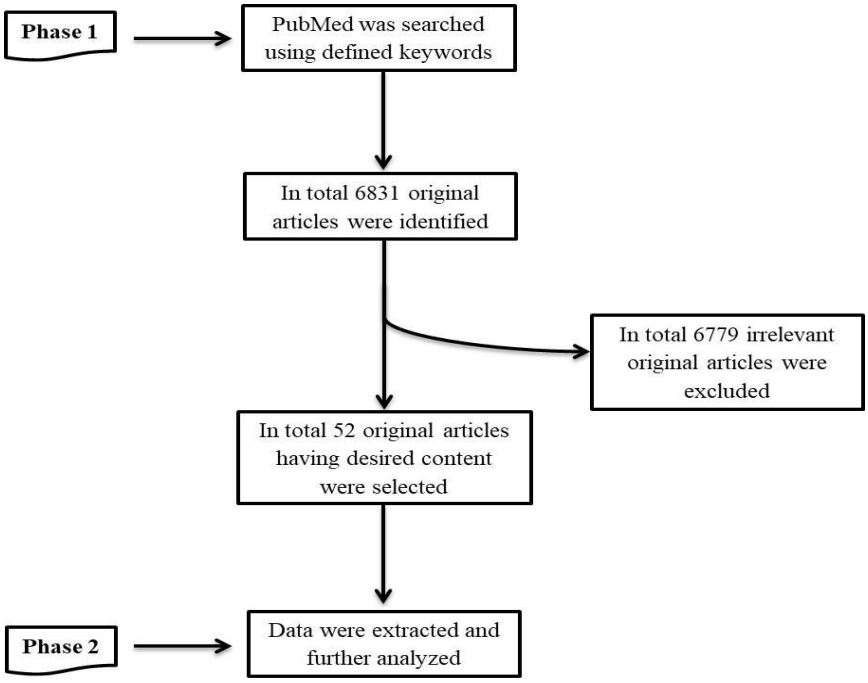

Figure 1: Overview of the methodology implemented during the present study.

Table 1: Summary of the Detection of HPV and positivity rate in normal and HNC samples relative to the different selected articles.

Studied Population	Technique used for viral genome detection	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 head and neck cancer samples screened	Percentage positivity of HPV in head and neck cancer samples (%)	References	P-value	CI (%)
United kingdom	*In situ* hybridization	16	30	0	0	0	28	21	(55)	--	--
Hungary	PCR	16	0	0	0	0	79	41.7	(54)	--	--
Netherlands	PCR, Southern blotting	16	7	0	0	0	10	50	(64)	--	--
Sweden	PCR, ELISA	16	2	0	0	0	34	11	(57)	--	--
	PCR	16	10	0	0	0	60	43	(52)	--	--
	PCR	16	320	4.4	0	0	85	20	(36)		
	PCR	16	0	0	0	0	87	78	(15)	--	--
	RT-PCR	16	0	0	0	0	203	49	(49)	--	--
	PCR	--	0	0	0	0	72	20.8	(38)	--	--
Brazil	PCR	16	0	0	0	0	90	3.33	(14)	--	--
Finland	ELISA	16	0	0	0	0	66	9	(32)	--	--
	In situ hybridization	16	0	0	21	28.6	51	11.8	(67)	--	--
Cuba	PCR, Southern blotting	16	25	16	29	82	33	48.5	(17)	0.05	--
Japan	PCR	16	70	4.2	0	0	74	16.2	(56)	<0.05	--
	PCR	16, 18	0	0	0	0	24	54.1	(40)	--	--
	PCR	16	0	0	0	0	66	36	(66)	--	--
	PCR, *in situ* hybridization	16, 18	0	0	0	0	101	57.4	(69)	--	--
	PCR, immunohistochemistry	16, 18	0	0	0	0	60	55.0	(68)	--	--
USA	PCR	16	12	16.7	0	0	44	25	(63)	--	--
	PCR	16	0	0	48	0	108	46	(65)	--	--
	In situ hybridization	--	0	0	0	0	174	56	(31)	--	--
	PCR	16	0	0	0	0	94	10.6	(62)	--	--
	PCR	16	0	0	0	0	409	5.9	(50)	--	95
Pakistan	PCR	16, 18	200	4	0	0	100	46	(16)	--	95
Norway	PCR	16	0	0	0	0	137	51.8	(35)	--	--
Venezuela	PCR	--	0	0	0	0	50	60	(60)	--	--
	In situ hybridization	--	0	0	0	0	50	70	(61)	--	--
Australia	Immunohistochemistry	--	0	0	0	0	95	61.1	(45)	--	--
Korea	PCR	16	25	4	0	0	36	36	(48)	--	--
China	PCR	18	189	3.1	0	0	178	14.04	(30)	--	--
	PCR	16, 18	68	2.94	0	0	200	27.5	(34)	--	95
	Quantum dots, *in situ* hybridization	16, 18	0	0	0	0	21	33.3	(70)	--	--
	PCR	16	0	0	0	0	1002	19.4	(47)	--	--
	PCR	16, 18	0	0	0	0	40	72.5	(27)	0.037	--
	PCR	16, 18	40	55	0	0	73	73.9	(71)	0.040	--
India	PCR	16, 18	0	0	0	0	91	41	(25)	--	--
	PCR, Hybrid Capture II test	16	0	0	0	0	222	32.4	(29)	--	--
	Immunohistochemistry	--	0	0	0	0	124	12.9	(39)	--	--
	PCR	16	46	0	0	0	60	48	(33)	--	--
	qPCR	16, 18	0	0	0	0	106	33	(58)	--	--
	Immunohistochemistry, *In situ* hybridization	--	0	0	0	0	42	31	(43)	--	--
	PCR	16, 18	0	0	0	0	110	33.6	(53)	--	--
	PCR	16, 18	0	0	0	0	34	70.6	(42)	--	--
	PCR	16, 18	0	0	0	0	45	77.3	(51)	--	--
	In situ hybridization	--	0	0	0	0	61	27.8	(59)	0.008	--
										9	
South Africa	PCR	18	0	0	0	0	59	11.8	(26)	--	--
Taiwan	PCR	16	0	0	0	0	92	75	(44)	--	--
France	PCR	16	0	0	0	0	52	62	(28)	--	--
Canada	PCR, Immunohistochemistry	16	0	0	0	0	55	58	(46)	--	--
Serbia	PCR	16	0	0	0	0	50	64	(41)	--	--
Mexico	PCR	16, 18	248	17.3	0	0	62	43.5	(24)	--	95
	PCR	16	0	0	0	0	51	42	(37)	--	--

PCR = Polymerase chain reaction