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Does Human Papillomavirus cause Human Lung Cancer? - Applying Bradford Hill Criteria Postulates

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Abstract

The role of human papillomavirus (HPV) in human lung cancer has already been extensively investigated with conflicting results. Although the researchers have attempted to establish a link between HPV and lung cancer through the statistical meta-analysis of the previous studies they failed to establish a more reliable link due to the shortcomings of the statistical meta-analysis**.** This study has been arranged to establish a link between HPV and lung cancer by applying the Bradford Hill criteria postulates. We identified population-wide studies relating to HPV with lung cancer through PubMed. Then we examined the available data on HPV prevalence in lung cancer, normal/benign samples, and applied the Bradford Hill criteria postulates on the available evidence to investigate the association between HPV and lung cancer. The Bradford-Hill criteria are very old, reliable, and widely accepted for establishing a link between the cause and disease. Additionally, to further enhance the reliability of our outcomes we have also evaluated the methodologies of the previous studies to address the possibility of false-negative and false-positive results. After a careful assessment of the extracted data against the postulates of Bradford Hill criteria, it was observed that previous studies do not fulfill all the major postulates of Bradford Hill criteria for causation including temporality, consistency, biological gradient, experiment, coherence, specificity, and analogy. Therefore, no causal relationship has been suggested between HPV and lung cancer. The results suggested HPV as a co-participant in the pathogenesis of lung cancer rather than a potential biomarker.

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Introduction

Cancer is the second leading cause of death worldwide after cardiovascular diseases (Kyu *et al.*, 2016) and has been classified into various types based on specific parameters (Idikio, 2011). Lung cancer is one of the most frequently reported cancer types and its incidence has been raised considerably worldwide in recent times. A total of 1.8 million new cases of lung cancer were reported worldwide in 2019 (Siegel *et al.*, 2019).

Lung cancer pathogenesis is a complex process which is driven by a blend of various genetic and environmental factors (Sarchianaki *et al.*, 2014) but tobacco consumption is assumed as the most critical factor associated with the development of lung cancer worldwide (Whiteman and Wilson, 2016), however, the epidemiologic data collected from different geographic regions worldwide have shown that approximately 25% lung cancer cases are not related to the tobacco consumption (Malhotra *et al.*, 2016a).

The increasing rate of lung cancer cases in non-smokers during the past few years (Sagerup *et al.*, 2014) has attracted scientists to investigate the risk factors other than tobacco consumptions in lung cancer development. As a result, numerous etiological factors such as occupational exposure, previous lung disease, diet, etc. have been investigated by the researchers (Malhotra *et al.*, 2016b).

Additionally, the role of human papillomavirus (HPV) infection in the development of various types of bronchogenic carcinomas has also been investigated. *Syrjänen et al.* were the first who proposed the possible role of HPV infection in lung cancer development in 1979.

Since then, numerous studies have been carried out for HPV DNA detection in lung cancers with varying HPV detection positivity ratios, the highest rate of HPV detection positivity was observed in Asian populations, while European countries comparatively reported a low HPV detection positivity in lung cancer patients (Tsyganov *et al.*, 2019).

In general, a statistical meta-analysis is usually preferred when establishing a correlation between the virus and the disease as compared to the single study.

This choice is based on the multiple advantages of the meta-analysis such as increased number of objects, greater diversity among the objects, and conclusion with a high level of evidence over the individual single study which has disadvantages like a small cohort of patients and conclusions with a low level of evidence. By keeping in view the inconsistencies in the HPV detection ratios in worldwide published studies, recently, researchers have analyzed the previously published studies relating HPV with lung cancer by the means of statistical meta-analysis to yield more useful pieces of information.

Xiong et al. (Xiong *et al.*, 2017) performed a meta-analysis of the available literature on HPV detection in lung cancer by searching various authentic research engines including MEDLINE (PubMed), Embase (OVID), and Web of Science. They obtained more than 30 case-control studies from different populations including European, Asian, and American. The results of their meta-analysis revealed that HPV infection significantly increased the risk of lung cancer development.

Similarly, another meta-analysis has been carried out by *Tsyganov et al.* in 2019 to investigate the role of HPV in lung cancer. In their meta-analysis, they retrieved the 26 case-control studies from various populations including Brazil, Korea, Greece, and Taiwan by searching PubMed, Web of Science, and Scopus databases. Their results revealed no significant association between HPV infection and lung cancer development.

Although evaluating the results of previous studies documenting the role of HPV in lung cancer development through statistical meta-analysis was a better choice than generalizing the results of an individual study, however, a statistical meta-analysis for molecular-level studies is not a more reliable method because of some serious limitations such as it

is not capable to evaluate the methodologies of the previous, so there is no way to assess the probability of false-negative and false-positive results.

In statistical meta-analysis researchers also do not consider the effect of heterogeneity-specific nature of the understudied populations on HPV detection. Furthermore, statistical meta-analysis often contributes to publication biasness, in which researchers do not pick studies that have no results even though they contain valuable information. By looking at the discrepancy in the outcomes of the previously published studies and significant shortcomings of the statistical meta-analysis, we evaluated the results of previous studies detecting HPV in lung cancer using the Bradford Hill criteria. These criteria are widely used and accepted worldwide over many years for establishing a causal relationship between a presumed cause and an observed effect of public health research (Fedak *et al*., 2015).

In the course of evaluation, we analyzed, whether or not these studies fulfill all the postulates of Bradford Hill criteria to declare a causal relationship between HPV and lung cancer. In addition, we also evaluated the methodologies used by the previous studies to address the possibility of false-positive and false-negative results. The outcomes of the present study will help to establish a more reliable causal relationship between HPV and lung cancer and determine the more appropriate treatment strategies for lung cancer patients.

Methodology

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

Literature search

All the relevant articles associating HPV with lung cancer were identified through the PubMed search database using the keywords: "Human papillomavirus" AND "Lung Cancer". We also defined "Papillomaviridae" AND "Non-small cell lung cancer" as medical subject headings (MeSH) terms. Mesh terms and keywords were combined during the search process. All the literature was searched available until August 2020, with the "Original Article" filter. In total 823 original articles were identified through the PubMed search engine.

Relevant data extraction

From 823 original articles, the 90 relevant articles were identified as having the desired information by initially reading the title, abstract, and then the complete article. Furthermore, a comprehensive table was constructed having all the required information from the selected relevant studies.

Evaluation of the results using the postulates of Bradford Hill criteria

Based on the extracted data, all the identified studies were carefully evaluated against the following Bradford Hill criteria postulates:

(1) Strength: larger the association, more probability of the causal relationship, (2) Temporality: cause must lead to the induction of an effect. If the delay is expected between the cause and effect, then the effect has to occur after the delay, (3) Consistency: different studies conducted by different researchers at different places with different sample size and reporting similar results increase the chances of the causal relationship between the cause and effect, (4) Plausibility: there should be plausible mechanism between the cause and effect, (5) Biological gradient: greater response is produced by the causative agent in response to the greater exposure. However, in some cases effect can be triggered by the mere presence of the factor while in other cases, greater exposure can lead to lower effect as well, (6) Experiment: the relationship between the cause and effect should be explained by the experiments and experiment should results in the reduction of effect when the causative agent is removed, (7) Coherence: causal relationship should not conflict with already known literature about the disease or exposure, (8) Specificity: causality is more likely if the effect has only one cause, (9) Analogy: previous evidence of the association between the cause and effect should

support the current statement for the causalrelationship. The assessment of each postulate was qualitative/descriptive, as there was an element of subjectivity in applying quantitative scoring. Evidence collected for each postulate is presented in Table 1 and the results section with a final judgment as to whether the postulate was fulfilled or not.

Results and discussion

Lung cancer is one of the most common types of cancer that infect millions of people worldwide each year. Although recent advancements in the diagnosis and treatment of lung cancer have helped to manage the disease still its prevalence is on a rise due to unknown underlying mechanisms (Miller, 2005).

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

Studied population	Name of the technique used for viral detection	Name of the target gene	Target strains	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 lung cancer samples screened	Percentage positivity of HPV in lung cancer samples (%)	References
China	PCR	--	6,11,16	6,11	0	0	0	0	49	14.3	(Xing *et al.*, 1993)
	PCR	--	11,16	11	0	0	0	0	49	14.3	(Liu *et al.*, 1994)
	PCR	--	6,11,16	6,11	0	0	0	0	49	14.3	(Xing *et al.*, 1994)
	PCR	--	16,18	16	0	0	0	0	50	32	(Li *et al.*, 1995)
	PCR	--	--	--	0	0	0	0	34	11.8	(Zhang *et al.*, 1995)
	PCR	--	--	--	0	0	0	0	40	55	(Da *et al.*, 1996)
	PCR	--	6,11,16	16	30	10	30	36.7	50	26	(Yang *et al.*, 1998)
	PCR	--	16,18	16,18	0	0	40	2.5	110	40	(Niyaz *et al.*, 2000)
	Immuno-histochemistry	--	16,18	16,18	34	5.9	0	0	73	31.5	(Fei *et al.*, 2006)
	PCR, in situ hybridization, Immuno-histochemistry	E6, E7	16,18	16,18	0	0	96	4.2	313	44.1	(Wang *et al.*, 2008)
	PCR	L1,E2,E6	--	6,16,18	0	0	0	0	109	39.4	(Yu *et al.*, 2009)
	Immuno-histochemistry	--	16,18	16,18	0	0	15	0	44	72.7	(Xu *et al.*, 2009)
	PCR	--	--	--	0	0	16	0	45	40	(Wang *et al.*, 2010)
	PCR, RT-PCR	E6, E2	6,16,18	16,18	91	23.1	0	0	170	44.1	(Yu *et al.*, 2013)
	PCR	L1	1,16,18		110	7.3	0	0	180	55.6	(Yu *et al.*, 2015)
	Liquid bead microarray antibody assays	HPyV, L1, E6, E7	--	--	0	0	217	0.9	183	4.4	(Colombara *et al.*, 2016)
	PCR	L1	--	----	0	0	83	7.2	83	8.4	(Xiong *et al.*, 2016)
	Immuno-histochemistry	E6, E7	--	--	0	0	55	1.8	95	44.2	(Fan *et al.*, 2016)
	PCR	E6, E7	16,18	18	0	0	54	3.7	72	45.8	(Lu *et al.*, 2016)
	PCR	L1	6,11,16, 18, 31, 33, 35, 39,42,43,44.45, 51, 52, 53, 56, 58, 59, 66, 68	16,18,42	0	0	140	11.4	140	9.2	(Fei he *et al.*, 2020)
USA	Immuno-histochemistry	--	6,11,16,18,31,33, 35	31,33, 35	0	0	0	0	79	8.9	(Yousem *et al.*, 1992)

	PCR	L1	6, 11, 16,18, 31, 33, 35	18	0	0	0	0	34	5.9	(Bohlmeyer *et al.*, 1998)
	PCR	L1	16,11	16	0	0	21	0	30	16.7	(Joh *et al.*, 2010)
	PCR	--	--	16,18	0	0	0	0	208	14.9	(Pillai *et al.*, 2013)
	Liquid bead microarray antibody assays	--	--	--	200	7.5	0	0	200	2	(Colombara *et al.*, 2015)
	PCR	L1	16, 18, 39, 44, 51. 52, 68	16, 44, 51, or 52	0	0	10	10	57	26.3	(Robinson *et al.*, 2016)
Germany	DNA hybridization	--	1, 2, 4, 8, 9, 10, 11, 13, 16 and 18.	16	0	0	0	0	24	4.2	(Stremlau *et al.*, 1985)
	PCR	L1, E1	2,7,10, 16,17,18,25,27,30,31, 32,34,35,40,42,45,4 9,53,56,57,60,6365	--	0	0	0	0	85	0	(Shamanin *et al.*, 1994)
	PCR, Immuno-histochemistry	L1	6, 11, 16, 18, and 33	--	0	0	0	0	38	0	(Welt *et al.*, 1997)
Japan	PCR, Southern blot hybridization	E6	16,18	18	0	0	0	0	121	13.2	(Ogura *et al.*, 1993)
	PCR	E6, E7	6,16,18,31,33,52b, and 58	--	0	0	0	0	47	0	(Szabó *et al.*, 1994)
	PCR	E6, E7	16,18,33	18	0	0	0	0	36	8.3	(Kinoshita *et al.*, 1995)
	PCR	--	16,18,33	18	0	0	0	0	8	12.5	(Sagawa *et al.*, 1995)
	Non-isotopic in situ hybridization, PCR	E6, E7	6,11,16,18,31,33,51	6,16,18	0	0	0	0	94	45.7	(Hirayasu *et al.*, 1996)
	Non-isotopic in situ hybridization, PCR	E6, E7	6,11,16,18,31,33,51	6,11,16, 18	0	0	0	0	23	78.3	(Tsuhako *et al.*, 1998)
	PCR	E6,E7	16,18,33	16	0	0	0	0	22	4.5	(Hiroshima *et al.*, 1999)
	PCR	E6, E7	6,11,16,18	6,11,16, 18	0	0	0	0	1109	79	(Miyagi *et al.*, 2000)
	PCR	E6, E7	6,11,16,18	16,18	0	0	0	0	121	33.9	(Miyagi *et al.*, 2001)
	PCR	L1, E2, E6	6,16,18,33	16	0	0	0	0	57	19.3	(Baba *et al.*, 2010)
	Multiplex PCR, Nested PCR	E1, L2, E6,E7	16,18,33	--	0	0	0	0	275	0	(Iwakawa *et al.*, 2010)
	PCR	L1,E1,E6,E7	16,18,33	--	0	0	0	0	42	16.7	(Kato *et al.*, 2012)
Greece	PCR	L1	6,11,16,18	16,18	0	0	26	0	52	61.5	(Papadopoulou *et al.*, 1998)
	Nested PCR	L1,E2,E6,E7	6,11,16,18,33	16	0	0	0	0	68	29.4	(Gorgoulis *et al.*, 1999)
	PCR	E7	16	16	0	0	16	68.8	26	100	(Krikelis *et al.*, 2010)
	Real-Time PCR	L1	6,11,16,18,33,59	16	0	0	16	0	100	19	(Sarchianaki *et al.*, 2014)
Italy	Real-Time PCR	E6, E7	16,18,31	16	0	0	38	0	38	21.1	(Ciotti *et al.*, 2006)
	Semi-nested PCR	L1	6,16,31	16	0	0	50	0	78	15.4	(Giuliani *et al.*, 2007)
	PCR	E1	16,18,31,33,45	31	68	0	0	0	89	13.5	(Carpagnano *et al.*, 2011)
	Real-Time PCR	E6, E7	16,18	--	0	0	0	0	399	0	(Koshiol *et al.*, 2011)
	PCR, immuno-histochemistry	L1, E6, E7	--	--	0	0	23	4.3	50	4	(Gatta *et al.*, 2012)
Canada	PCR, Immuno-	L1	16, 18, 31, 33, 35, 45,	16	0	0	0	0	336	1.5	(Yanagawa *et*

	histochemistry		51, 52, 56, 58, and 66								*al.*, 2013)
Netherlands	PCR	L1, E7	6,11,16,18,26, 31,33,34, 35,39,40, 42, 43, 44,45,51,52,53, 54, 55, 56,57,58,59, 61, 66,68,70, 71, 72, 73, 81, 83, 84	--	0	0	0	0	211	0	(van Boerdonk *et al.*, 2013)
Norway	PCR	L1, E6	6,11,16,18,33	16	0	0	0	0	75	49.3	(Hennig *et al.*, 1999b)
	PCR	--	--	--	0	0	0	0	2	50	(Hennig *et al.*, 1999a)
	PCR	E6,E7	6,11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68	16, 33, 66	0	0	13	0	334	3.9	(Sagerup *et al.*, 2014)
Poland	PCR	--	--	--	0	0	0	0	40	12.5	(Miasko *et al.*, 2001)
Turkey	Nonisotopic in situ hybridization	--	6,11, 16, 18, 31, 33	16,18	0	0	0	0	26	11.5	(Kaya *et al.*, 2001)
	PCR	L1	--	--	0	0	0	0	40	5	(Zafer *et al.*, 2004)
	PCR	L1	16.18	--	0	0	87	0	65	1.5	(Buyru *et al.*, 2008)
Finland	in situ hybridization	--	6, 11, 16, 18, and 30	16	0	0	0	0	131	9.2	(Syrjänen *et al.*, 1989)
	Immuno-histochemistry, PCR	L1	6, 11, 16, 18, 31	31	0	0	0	0	22	77.3	(Nuorva *et al.*, 1995)
	In situ hybridisation, PCR	L1	6, 11, 16, 18, 31, 33	--	0	0	0	0	43	30.2	(Soini *et al.*, 1996)
	ELISA	--	16.18	--	930	23.7	0	0	311	21.5	(Simen-Kapeu *et al.*, 2010)
France	In situ hybridisation,	--	6, 11, 16, 18	11	0	0	10	0	33	18.2	(Bejui-Thivolet *et al.*, 1990)
	Nested PCR	E6 or E7	6,11,16,18	6,11	0	0	0	0	31	16.1	(Thomas *et al.*, 1995)
	Nested PCR	--	6,11,16,18	6,11	0	0	0	0	31	16.1	(Thomas *et al.*, 1996)
	Hybridization technique (Hybrid Capture II assay)	--	--	--	0	0	0	0	185	2.7	(Clavel *et al.*, 2000)
	Immuno-histochemistry	--	6, 11, 16, 18, 31, 33, 42, 51, 52, 56 and 58	--	0	0	0	0	122	0	(Brouchet *et al.*, 2005)
	PCR	E6	6,11,16, 18, 26, 31, 33, 35, 39,40, 42,54, 55, 57, 45, 51, 52, 53, 56, 58, 59, 61, 62, 64, 66, 67, 68, 70,71,72, 81 73,82, 83,84, CP6108	--	0	0	0	0	318	1.4	(Coissard *et al.*, 2005)
Croatia	PCR	E6	16, 18, 33	16, 18, 33	0	0	0	0	84	3.6	(Branica *et al.*, 2010)
Australia	Immuno-histochemistry	--	6, 11, 16, and 18	--	0	0	0	0	5	40	(Kulski *et al.*, 1990)
	PCR	E6-E7, L1	--	--	0	0	104	0	104	1.9	(Fong *et al.*, 1995)
India	PCR	L1	16,18	18	0	0	40	0	40	5	(Jain *et al.*, 2005)
	PCR	--	16, 18, 31, 33 ,45	16	75	0	0	0	73	6.8	(Gupta *et al.*, 2016)
Iran	Nested PCR	L1	--	16,18	90	8.9	0	0	141	23.4	(Nadji *et al.*, 2007)
UK	PCR	E1,L2	7, 6b, 11, 16, 18	6,11	0	0	26	11.5	40	7.5	(al-Ghamdi *et*

											al., 1995)
Korea	PCR	E6	16, 18, 33	33	0	0	0	0	112	45.5	(Park *et al.*, 2007)
Singapore	In situ hybridization	--	16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 66	--	0	0	0	0	110	0	(Lim *et al.*, 2009)
Taiwan	Nested PCR	L1	16,18	16,18	60	26.1	0	0	141	54.6	(Cheng *et al.*, 2001)
	Nested PCR	L1	16,18	16,18	174	12.6	0	0	149	47.7	(Chiou *et al.*, 2003)
	Nested PCR	L1	--	--	0	0	0	0	141	28.4	(Cheng *et al.*, 2004)
	PCR	L1	--	--	0	0	0	0	57	50.9	(Lin *et al.*, 2005)
	PCR, Immuno-histochemistry	--	--	--	0	0	0	0	166	54.8	(Wu *et al.*, 2005)
	Nested PCR	L1	--	--	0	0	0	0	153	55.5	(Wang *et al.*, 2006)
	Nested PCR	L1, E6	--	--	0	0	0	0	210	35.2	(Wang *et al.*, 2014)
Argentina	PCR, Immunohistochemistry	--	--	--	0	0	0	0	40	25	(Falcone *et al.*, 2017)
Brazil	PCR	L1, E6, E7	--	--	0	0	0	0	63	52.4	(de Oliveira *et al.*, 2018)
	RT-PCR	E7	--	--	0	0	0	0	62	0	(Silva *et al.*, 2019)
Chile	PCR	L1	--	--	0	0	0	0	69	29	(Aguayo *et al.*, 2007)
Czech	Real-time PCR	E6	--	--	0	0	0	0	80	0	(Jaworek *et al.*, 2020)

To date, various individual studies have been carried out worldwide to find the state of association between HPV and lung cancer to further uncover the molecular pathways regulating HPV but their results are conflicting. Besides, the statistical meta-analysis was also used by the researchers to analyze the previous individual studies for generating a more meaningful association between HPV and lung cancer but due to the shortcomings of statistical meta-analysis, researchers once again failed to establish a more reliable causal relationship between HPV and lung cancer. Hence, the current study was arranged to explore the association between HPV and lung cancer by applying the Bradford hill criteria postulates to the available literature associating HPV with lung cancer.

In total 90 relevant original articles (Stremlau *et al.*, 1985; Syrjänen *et al.*, 1989; Bejui-Thivolet *et al.*, 1990; Kulski *et al.*, 1990; Yousem *et al.*, 1992; Ogura *et al.*, 1993; Xing *et al.*, 1993; Liu *et al.*, 1994; Shamanin *et al.*, 1994; Szabó *et al.*, 1994; Xing *et al.*, 1994; al-Ghamdi *et al.*, 1995; Fong *et al.*, 1995; Kinoshita *et al.*, 1995; Li *et al.*, 1995; Nuorva *et al.*, 1995; Sagawa *et al.*, 1995; Thomas *et al.*, 1995; Zhang *et al.*, 1995; Da *et al.*, 1996; Hirayasu *et al.*, 1996; Soini *et al.*, 1996; Thomas *et al.*, 1996; Welt *et al.*, 1997; Bohlmeyer *et al.*, 1998; Papadopoulou *et al.*, 1998; Tsuhako *et al.*, 1998; Yang *et al.*, 1998; Gorgoulis *et al.*, 1999; Hennig *et al.*, 1999a; Hennig *et al.*, 1999b; Hiroshima *et al.*, 1999; Clavel *et al.*, 2000; Miyagi *et al.*, 2000; Niyaz *et al.*, 2000; Cheng *et al.*, 2001; Kaya *et al.*, 2001; Miasko *et al.*, 2001; Miyagi *et al.*, 2001; Chiou *et al.*, 2003; Cheng *et al.*, 2004; Zafer *et al.*, 2004; Brouchet *et al.*, 2005; Coissard *et al.*, 2005; Jain *et al.*, 2005; Lin *et al.*, 2005; Wu *et al.*, 2005; Ciotti *et al.*, 2006; Fei *et al.*, 2006; Wang *et al.*, 2006; Aguayo *et al.*, 2007; Giuliani *et al.*, 2007; Nadji *et al.*, 2007; Park *et al.*, 2007; Buyru *et al.*, 2008; Wang *et al.*, 2008; Lim *et al.*, 2009; Xu *et al.*, 2009; Yu *et al.*, 2009; Baba *et al.*, 2010; Branica *et al.*, 2010; Iwakawa *et al.*, 2010; Joh *et al.*, 2010; Krikelis *et al.*, 2010; Simen-Kapeu *et al.*, 2010; Wang *et al.*, 2010; Carpagnano *et al.*, 2011; Koshiol *et al.*, 2011; Gatta *et al.*, 2012; Kato *et al.*, 2012; Pillai *et al.*, 2013; van Boerdonk *et al.*, 2013; Yanagawa *et al.*, 2013; Yu *et al.*, 2013; Sagerup *et al.*, 2014; Sarchianaki *et al.*,

2014; Wang *et al.*, 2014; Colombara *et al.*, 2015; Yu *et al.*, 2015; Colombara *et al.*, 2016; Fan *et al.*, 2016; Gupta *et al.*, 2016; Lu *et al.*, 2016; Robinson *et al.*, 2016; Xiong *et al.*, 2016; Falcone *et al.*, 2017; de Oliveira *et al.*, 2018; Silva *et al.*, 2019; Jaworek *et al.*, 2020; *Fei he et al.*, 2020) (Table 1; Fig. 2) were found on PubMed which investigated the association of HPV with lung cancer in 25 different populations.

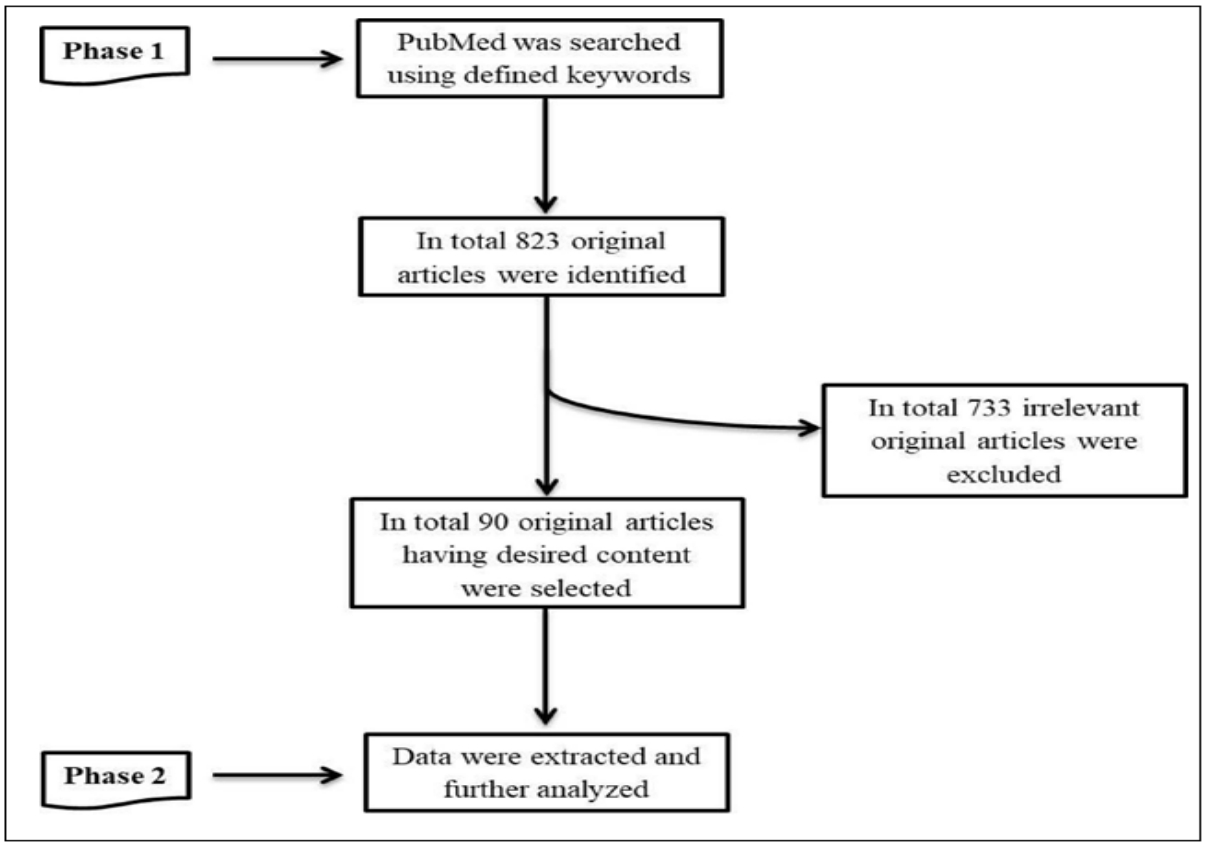

Fig. 1. Overview of the methodology implemented during the present study**.**

Table 1 enlists all these articles and contains essential information extracted from these articles including details of the studied population, techniques used for HPV detection, name of the target gene, the identified strains of HPV, the most prevalent identified stain, number (No) of screened samples (normal, benign and diseased) with their respective identified population-wise positivity ratios.

Out of all the 90 studies, in total only n = 34 studies (Bejui-Thivolet *et al.*, 1990; al-Ghamdi *et al.*, 1995; Fong *et al.*, 1995; Papadopoulou *et al.*, 1998; Yang *et al.*, 1998; Niyaz *et al.*, 2000; Cheng *et al.*, 2001; Chiou *et al.*, 2003; Jain *et al.*, 2005; Ciotti *et al.*, 2006; Fei *et al.*, 2006; Giuliani *et al.*, 2007; Nadji *et al.*, 2007; Buyru *et al.*, 2008; Wang *et al.*, 2008; Xu *et al.*, 2009; Joh *et al.*, 2010; Krikelis *et al.*, 2010; Simen-Kapeu *et al.*, 2010; Wang *et al.*, 2010; Carpagnano *et al.*, 2011; Gatta *et al.*, 2012; Yu *et al.*, 2013; Sagerup *et al.*, 2014; Sarchianaki *et al.*, 2014; Colombara *et al.*, 2015; Yu *et al.*, 2015; Colombara *et al.*, 2016; Fan *et al.*, 2016; Gupta *et al.*, 2016; Lu *et al.*, 2016; Robinson *et al.*, 2016; Xiong *et al.*, 2016; *Fei he et al.*, 2020) were the case-control studies in which normal, benign and lung cancer samples were screened while others were not (Stremlau *et al.*, 1985; Syrjänen *et al.*, 1989; Kulski *et al.*, 1990; Yousem *et al.*, 1992; Ogura *et al.*, 1993; Xing *et al.*, 1993; Liu *et al.*, 1994; Shamanin *et al.*, 1994; Szabó *et al.*, 1994; Xing *et al.*, 1994; Kinoshita *et al.*, 1995; Li *et al.*, 1995; Nuorva *et al.*, 1995; Sagawa *et al.*, 1995; Thomas *et al.*, 1995; Zhang *et al.*, 1995; Da *et al.*, 1996; Hirayasu *et al.*, 1996; Soini *et al.*, 1996; Thomas *et al.*, 1996; Welt *et al.*, 1997; Bohlmeyer *et al.*, 1998; Tsuhako *et al.*, 1998; Gorgoulis *et al.*, 1999; Hennig *et al.*, 1999a; Hennig *et al.*, 1999b; Hiroshima *et al.*, 1999; Clavel *et*

al., 2000; Miyagi *et al.*, 2000; Kaya *et al.*, 2001; Miasko *et al.*, 2001; Miyagi *et al.*, 2001; Cheng *et al.*, 2004; Zafer *et al.*, 2004; Brouchet *et al.*, 2005; Coissard *et al.*, 2005; Lin *et al.*, 2005; Wu *et al.*, 2005; Wang *et al.*, 2006; Aguayo *et al.*, 2007; Park *et al.*, 2007; Lim *et al.*, 2009; Yu *et al.*, 2009; Baba *et al.*, 2010; Branica *et al.*, 2010; Iwakawa *et al.*, 2010; Koshiol *et al.*, 2011; Kato *et al.*, 2012; Pillai *et al.*, 2013; van Boerdonk *et al.*, 2013; Yanagawa *et al.*, 2013; Wang *et al.*, 2014; Falcone *et al.*, 2017; de Oliveira *et al.*, 2018; Silva *et al.*, 2019; Jaworek *et al.*, 2020).

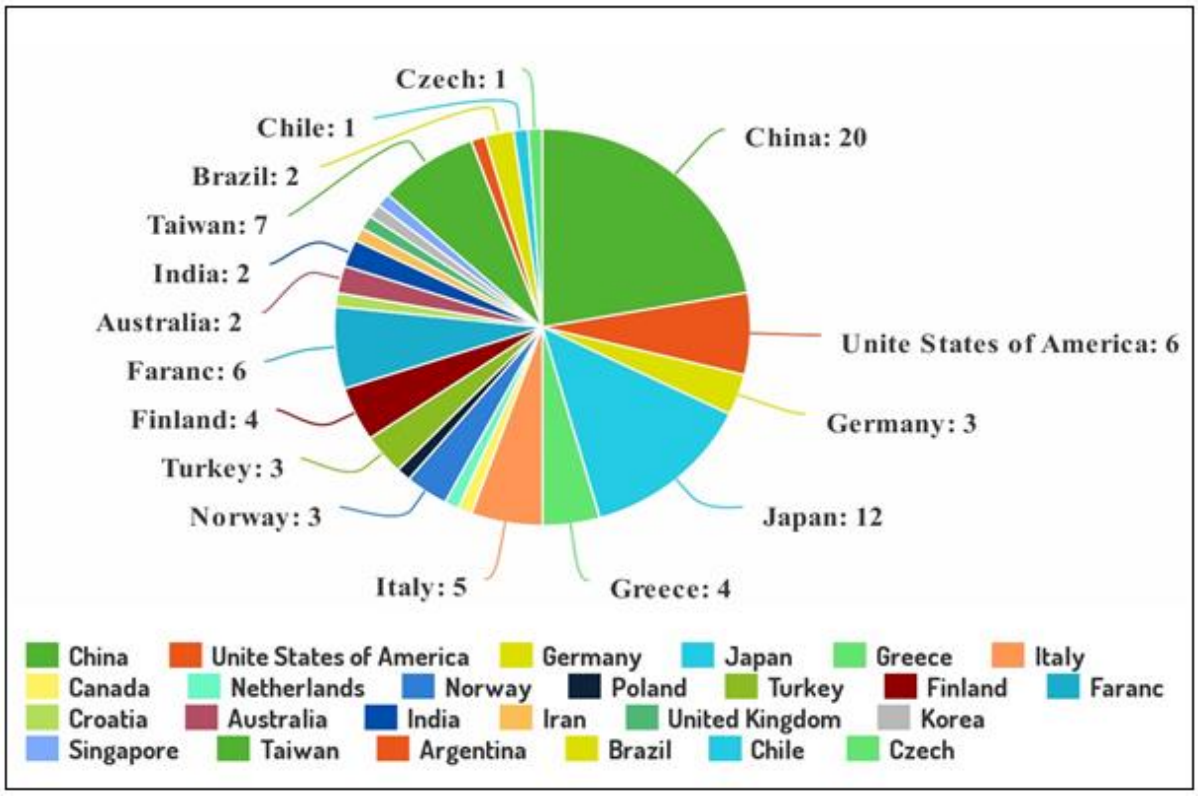

Fig. 2. Comparison graph between the numbers (No) of the studies carried out in each population on HPV in lung cancer.

The positivity ratio of HPV detection in lung cancer samples was varied population-wise from 0% (Shamanin *et al.*, 1994; Szabó *et al.*, 1994; Welt *et al.*, 1997; Brouchet *et al.*, 2005; Lim *et al.*, 2009; Iwakawa *et al.*, 2010; Koshiol *et al.*, 2011; van Boerdonk *et al.*, 2013; Silva *et al.*, 2019; Jaworek *et al.*, 2020) to 100% (Krikelis *et al.*, 2010) in all the 90 studies. While, the positivity ratio of HPV detection in normal and adjacent/benign samples was varied from 0% (Carpagnano *et al.*, 2011; Gupta *et al.*, 2016) to 26.1% (Cheng *et al.*, 2001) and 0% (Bejui-Thivolet *et al.*, 1990; Fong *et al.*, 1995; Papadopoulou *et al.*, 1998; Ciotti *et al.*, 2006; Giuliani *et al.*, 2007; Buyru *et al.*, 2008; Xu *et al.*, 2009; Joh *et al.*, 2010; Wang *et al.*, 2010; Sagerup *et al.*, 2014; Sarchianaki *et al.*, 2014) to 68.8% (Krikelis *et al.*, 2010), respectively. Possible reasons for such population-specific inequalities in HPV detection could be non-modifiable factors such as genetic makeup and socially controllable factors like health-seeking behavior and differential access to the health facilities.

The careful evaluation of the results of identified studies through Bradford hill criteria showed that all the studies failed to completely fulfill all the major postulates of Bradford hill criteria.

As an example, if we look at the HPV detection positivity ratios in the Chinese population the results are inconsistent. Out of 20, a total of 2 (Yang *et al.*, 1998; *Fei he et al.*, 2020) studies have reported the

higher HPV detection positivity ratios in adjacent/benign samples (36.7% and 11.4%) as compared to the lung cancer samples (26% and 9.2%). Hence, the documented results in Chinese population do not fulfill the major Bradford hill criteria postulates including strength; according to this postulate the relationship will be more casual if the association is greater, however, in total 2 studies (Yang *et al.*, 1998; *Fei he et al.*, 2020) also shown the greater association of HPV with adjacent/benign samples as compared to the lung cancer samples so this postulate was not fullfilled completely in Chinese population, temporality; according to this postulate a cause must lead to the induction of effect, however, in Chinses population HPV was also detected in adjacent/benign controls even with a higher detection postivity ratios as mentioned in (Yang *et al.*, 1998; *Fei he et al.*, 2020), so this postulate was not fulfilled, consistency; according to this postulate the more consistent results of the different studies the more chances of a causal relationship between cause and effect but any such situation was not observed in Chinese population where few studies reported the higher HPV presence in adjacent/benign samples (Yang *et al.*, 1998; *Fei he et al.*, 2020) as compared to the lung cancer samples so this postulate was not fulfilled. Similarly, biological gradient, experiment, coherence, specificity, and analogy postulates have also been not fulfilled by Chinese as well as other populations which are likely based on similar parameters. However, the strength postulate was an exception that was fulfilled in a few populations like Greece, Norway, Turkey, France, Australia, India, Iran and Taiwan.

Hence, we suggested that HPV acts as a co-participant in the development of lung cancer rather than having a causal relationship, so based on our results it is 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 lung cancer by affecting the body's immune system. Furthermore, the evaluation of methodologies utilized by previous studies revealed that Polymerase chain reaction (PCR) technique was employed by most of the studies (Ogura *et al.*, 1993; Xing *et al.*, 1993; Liu *et al.*, 1994; Shamanin *et al.*, 1994; Szabó *et al.*, 1994; Xing *et al.*, 1994; al-Ghamdi *et al.*, 1995; Fong *et al.*, 1995; Kinoshita *et al.*, 1995; Li *et al.*, 1995; Nuorva *et al.*, 1995; Sagawa *et al.*, 1995; Thomas *et al.*, 1995; Zhang *et al.*, 1995; Da *et al.*, 1996; Hirayasu *et al.*, 1996; Soini *et al.*, 1996; Thomas *et al.*, 1996; Welt *et al.*, 1997; Bohlmeyer *et al.*, 1998; Papadopoulou *et al.*, 1998; Tsuhako *et al.*, 1998; Yang *et al.*, 1998; Gorgoulis *et al.*, 1999; Hennig *et al.*, 1999a; Hennig *et al.*, 1999b; Hiroshima *et al.*, 1999; Miyagi *et al.*, 2000; Niyaz *et al.*, 2000; Cheng *et al.*, 2001; Miasko *et al.*, 2001; Miyagi *et al.*, 2001; Chiou *et al.*, 2003; Cheng *et al.*, 2004; Zafer *et al.*, 2004; Coissard *et al.*, 2005; Jain *et al.*, 2005; Lin *et al.*, 2005; Wu *et al.*, 2005; Ciotti *et al.*, 2006; Wang *et al.*, 2006; Aguayo *et al.*, 2007; Giuliani *et al.*, 2007; Nadji *et al.*, 2007; Park *et al.*, 2007; Buyru *et al.*, 2008; Wang *et al.*, 2008; Yu *et al.*, 2009; Baba *et al.*, 2010; Branica *et al.*, 2010; Iwakawa *et al.*, 2010; Joh *et al.*, 2010; Krikelis *et al.*, 2010; Simen-Kapeu *et al.*, 2010; Wang *et al.*, 2010; Carpagnano *et al.*, 2011; Koshiol *et al.*, 2011; Gatta *et al.*, 2012; Kato *et al.*, 2012; Pillai *et al.*, 2013; van Boerdonk *et al.*, 2013; Yanagawa *et al.*, 2013; Yu *et al.*, 2013; Sagerup *et al.*, 2014; Sarchianaki *et al.*, 2014; Wang *et al.*, 2014; Yu *et al.*, 2015; Gupta *et al.*, 2016; Lu *et al.*, 2016; Robinson *et al.*, 2016; Xiong *et al.*, 2016; Falcone *et al.*, 2017; de Oliveira *et al.*, 2018; Silva *et al.*, 2019; Jaworek *et al.*, 2020; *Fei he et al.*, 2020) to detect the presence of HPV in the normal, adjacent/benign and diseased samples using L1, E6, and E7 gene specific primers which specifically target (6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 45, and 51–59) subtypes of HPV, from them, additionally, few studies also employed the second techniques including Immunohistochemistry (Nuorva *et al.*, 1995; Welt *et al.*, 1997; Wu *et al.*, 2005; Wang *et al.*, 2008; Gatta *et al.*, 2012; Yanagawa *et al.*, 2013; Falcone *et al.*, 2017), In situ hybridization (Soini *et al.*, 1996; Wang *et al.*, 2008) and Southern blotting (Ogura *et al.*, 1993) to validate their PCR results. Few studies also used immunohistochemistry

(Kulski *et al.*, 1990; Yousem *et al.*, 1992; Brouchet *et al.*, 2005; Fei *et al.*, 2006; Xu *et al.*, 2009; Fan *et al.*, 2016), Liquid bead microarray antibody assay (Colombara *et al.*, 2015; Colombara *et al.*, 2016), ELISA (Simen-Kapeu *et al.*, 2010) and *In-situ* hybridization (Bejui-Thivolet *et al.*, 1990; Hirayasu *et al.*, 1996; Tsuhako *et al.*, 1998; Kaya *et al.*, 2001; Lim *et al.*, 2009), for the intial detection of HPV and they did not validate their results through any other technique. However, limitations and some of the major issues related to methodologies used in the included studies have been discussed below.

Possible causes of the false-positive results

Most of the studies that we summarized used PCR technique (Ogura *et al.*, 1993; Xing *et al.*, 1993; Liu *et al.*, 1994; Shamanin *et al.*, 1994; Szabó *et al.*, 1994; Xing *et al.*, 1994; al-Ghamdi *et al.*, 1995; Fong *et al.*, 1995; Kinoshita *et al.*, 1995; Li *et al.*, 1995; Nuorva *et al.*, 1995; Sagawa *et al.*, 1995; Thomas *et al.*, 1995; Zhang *et al.*, 1995; Da *et al.*, 1996; Hirayasu *et al.*, 1996; Soini *et al.*, 1996; Thomas *et al.*, 1996; Welt *et al.*, 1997; Bohlmeyer *et al.*, 1998; Papadopoulou *et al.*, 1998; Tsuhako *et al.*, 1998; Yang *et al.*, 1998; Gorgoulis *et al.*, 1999; Hennig *et al.*, 1999a; Hennig *et al.*, 1999b; Hiroshima *et al.*, 1999; Miyagi *et al.*, 2000; Niyaz *et al.*, 2000; Cheng *et al.*, 2001; Miasko *et al.*, 2001; Miyagi *et al.*, 2001; Chiou *et al.*, 2003; Cheng *et al.*, 2004; Zafer *et al.*, 2004; Coissard *et al.*, 2005; Jain *et al.*, 2005; Lin *et al.*, 2005; Wu *et al.*, 2005; Ciotti *et al.*, 2006; Wang *et al.*, 2006; Aguayo *et al.*, 2007; Giuliani *et al.*, 2007; Nadji *et al.*, 2007; Park *et al.*, 2007; Buyru *et al.*, 2008; Wang *et al.*, 2008; Yu *et al.*, 2009; Baba *et al.*, 2010; Branica *et al.*, 2010; Iwakawa *et al.*, 2010; Joh *et al.*, 2010; Krikelis *et al.*, 2010; Simen-Kapeu *et al.*, 2010; Wang *et al.*, 2010; Carpagnano *et al.*, 2011; Koshiol *et al.*, 2011; Gatta *et al.*, 2012; Kato *et al.*, 2012; Pillai *et al.*, 2013; van Boerdonk *et al.*, 2013; Yanagawa *et al.*, 2013; Yu *et al.*, 2013; Sagerup *et al.*, 2014; Sarchianaki *et al.*, 2014; Wang *et al.*, 2014; Yu *et al.*, 2015; Gupta *et al.*, 2016; Lu *et al.*, 2016; Robinson *et al.*, 2016; Xiong *et al.*, 2016; Falcone *et al.*, 2017; de Oliveira *et al.*, 2018; Silva *et al.*, 2019; Jaworek *et al.*, 2020; *Fei he et al.*, 2020) for the detection of HPV and none of them utilized second technique to confirm their positive results of PCR, except n = 03 studies (Ogura *et al.*, 1993; Soini *et al.*, 1996; Wang *et al.*, 2008) which utilized immunohistochemistry (Wang *et al.*, 2008), *In-situ* hybridization (Wang *et al.*, 2008), Southern blotting (Ogura *et al.*, 1993), and the results of their second technique have deviated from the first one in two studies (Ogura *et al.*, 1993; Wang *et al.*, 2008). Expression of the p16, Bcl-2, p53, EGFR and some other genes could be used as a surrogate biomarker in HPV infected lung cancer patients. Along with HPV detection, these surrogate biomarkers were also analyzed by some studies (Niyaz *et al.*, 2000; Wu *et al.*, 2005; Gatta *et al.*, 2012; Yanagawa *et al.*, 2013; Yu *et al.*, 2013; Robinson *et al.*, 2016; Falcone *et al.*, 2017) to further validate their findings, out of which have validated their findings by analyzing these surrogate biomarkers while the other studies (Gatta *et al.*, 2012; Falcone *et al.*, 2017) were failed to validate their findings the surrogate biomarkers. Such deviations in the results of previous studies raise a big question mark on the selection of appropriate technologies 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 lung cancer samples (Stremlau *et al.*, 1985; Syrjänen *et al.*, 1989; Kulski *et al.*, 1990; Yousem *et al.*, 1992; Ogura *et al.*, 1993; Xing *et al.*, 1993; Liu *et al.*, 1994; Shamanin *et al.*, 1994; Szabó *et al.*, 1994; Xing *et al.*, 1994; Kinoshita *et al.*, 1995; Li *et al.*, 1995; Nuorva *et al.*, 1995; Sagawa *et al.*, 1995; Thomas *et al.*, 1995; Zhang *et al.*, 1995; Da *et al.*, 1996; Hirayasu *et al.*, 1996; Soini *et al.*, 1996; Thomas *et al.*, 1996; Welt *et al.*, 1997; Bohlmeyer *et al.*, 1998; Tsuhako *et al.*, 1998; Gorgoulis *et al.*, 1999; Hennig *et al.*, 1999a; Hennig *et al.*, 1999b; Hiroshima *et al.*, 1999; Clavel *et al.*, 2000; Miyagi *et al.*, 2000; Kaya *et al.*, 2001; Miasko *et al.*, 2001; Miyagi *et al.*, 2001; Cheng *et al.*, 2004; Zafer *et al.*, 2004; Brouchet *et al.*, 2005; Coissard *et al.*, 2005; Lin *et al.*, 2005; Wu *et*

al., 2005; Wang *et al.*, 2006; Aguayo *et al.*, 2007; Park *et al.*, 2007; Lim *et al.*, 2009; Yu *et al.*, 2009; Baba *et al.*, 2010; Branica *et al.*, 2010; Iwakawa *et al.*, 2010; Koshiol *et al.*, 2011; Kato *et al.*, 2012; Pillai *et al.*, 2013; van Boerdonk *et al.*, 2013; Yanagawa *et al.*, 2013; Wang *et al.*, 2014; Falcone *et al.*, 2017; de Oliveira *et al.*, 2018; Silva *et al.*, 2019; Jaworek *et al.*, 2020) and did not allow us to compare their results with normal or adjacent/benign controls. However, most of the studies also analyzed the normal and adjacent/benign tissues along with lung cancer samples and comparison of their results demonstrated that HPV detection positivity ratios in lung cancer samples were higher in (Bejui-Thivolet *et al.*, 1990; al-Ghamdi *et al.*, 1995; Fong *et al.*, 1995; Papadopoulou *et al.*, 1998; Niyaz *et al.*, 2000; Cheng *et al.*, 2001; Chiou *et al.*, 2003; Jain *et al.*, 2005; Ciotti *et al.*, 2006; Fei *et al.*, 2006; Giuliani *et al.*, 2007; Nadji *et al.*, 2007; Buyru *et al.*, 2008; Wang *et al.*, 2008; Xu *et al.*, 2009; Joh *et al.*, 2010; Krikelis *et al.*, 2010; Wang *et al.*, 2010; Carpagnano *et al.*, 2011; Yu *et al.*, 2013; Sagerup *et al.*, 2014; Sarchianaki *et al.*, 2014; Yu *et al.*, 2015; Colombara *et al.*, 2016; Fan *et al.*, 2016; Gupta *et al.*, 2016; Lu *et al.*, 2016; Robinson *et al.*, 2016; Xiong *et al.*, 2016) studies while lower in (Yang *et al.*, 1998; Simen-Kapeu *et al.*, 2010; Gatta *et al.*, 2012; Colombara *et al.*, 2015; *Fei he et al.*, 2020) studies as compared to the normal and adjacent/benign controls. However, none of the studies has reported the association of HPV with specific lung cancer subtype and histologic grade.

Conclusion

The results of this comprehensive review are controversial. They failed to prove the causal relationship between HPV and lung cancer rather suggested it as a co-participant in the pathogenesis of lung cancer. However, due to the limitations of the methodologies used by the previous studies to detect the presence of HPV in lung cancer, additional experiments are recommended to prove the HPV etiology in lung cancer.

Conflict of interest

None.

Acknowledgment

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