Advances in Infectious Diseases, **2013, 3, 17-34**
http://dx.doi.org/10.4236/aid.2013.31003 Published Online March 2013 (http://www.scirp.org/journal/aid)

The Relationship between Human Papillomavirus and Oesophageal Squamous Cell Carcinoma in China—A Review of the Evidence*

Surabhi S. Liyanage[1#], Qian Li[2], Yang Zheng[3], Holly Seale[1], Philip J. Crowe[4], Anthony T. Newall[1], Bayzidur Rahman[1], Eva Segelov[5], Chenxu Qu[6], Fanghui Zhao[6], Junfeng Liu[2], Zhanhai Gao[1], Weixian Shi[3], Peng Yang[3], Aye Moa[1], Chandini Raina MacIntyre[1]

[1]School of Public Health and Community Medicine, The University of New South Wales Medicine, The University of New South Wales, Sydney, Australia; [2]Department of Surgery, Fourth Hospital, Shijiazhuang, China; [3]Centre for Disease Control and Prevention, Beijing, China; [4]Department of Surgery, Prince of Wales Hospital Clinical School, The University of New South Wales, Sydney, Australia; [5]Department of Medicine, St. Vincent's Hospital Clinical School, The University of New South Wales, Sydney, Australia; [6]Department of Cancer Epidemiology, Cancer Institute, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China.
Email: #Surabhi_liyanage@hotmail.com

Received December 6th, 2012; revised January 7th, 2013; accepted February 9th, 2013

ABSTRACT

Background: China has one of the highest incidence rates of oesophageal cancer in the world. The role of human papillomavirus (HPV) has been extensively researched in oesophageal squamous cell carcinoma (OSCC) with indeterminate results. The majority of these studies have been conducted in the Chinese population. Evidence for a definitive HPV-OSCC association could potentially support prophylactic vaccination in target populations, highlighting the need for ongoing investigation. The aim of this review is to summarise the findings of HPV DNA in OSCC tissue in Chinese subjects, with a view to informing further research in this area. **Methods:** A systematic literature search of the Chinese National Knowledge Infrastructure (CNKI) database, Medline, Embase and PubMed was conducted for all studies in English and Chinese language, examining OSCC tissue for HPV DNA in China. Reference lists of retrieved articles were reviewed and hand searches of relevant, key journals were conducted, to source articles which were not electronically indexed. Sixty-four studies met our selection criteria. Data from case-control and cross-sectional studies were analysed separately for any HPV-OSCC association, using the Epi Info™ 3.5.3 software program. **Results:** From all studies conducted in the Chinese population, 2166/5953 (36%) of all OSCC tissue and 478/1684 (28%) of healthy control tissue, tested positive for HPV. We found that 11/16 case-control and cross-sectional studies had a statistically significant crude odds ratio, which supported a potential HPV-OSCC association. The largest study, carried out in the high incidence County of Anyang in Henan Province, reported 207/265 (78%) OSCC tissues testing positive for HPV DNA against 203/357 (57%) controls and had an unadjusted odds ratio of 2.71 (p-value < 0.0001). **Conclusion:** A rigorous meta-analysis would improve interpretation of the data and a well-designed large-scale case-control study is warranted. If a link is found between HPV and OSCC, prophylactic HPV vaccines could be of significant benefit in China.

Keywords: Human Papillomavirus; Oesophageal Carcinoma; Squamous Cell Carcinoma; HPV Vaccine; China

1. Background

The role of human papillomavirus (HPV) as a potential aetiological factor in oesophageal squamous cell carcinoma (OSCC) has been debated for the last three decades [1]. The pathogenesis of HPV in cervical cancer is well established and the International Agency on Research on Cancer (IARC) has accepted the role of HPV in several head and neck cancers [2]. However, evidence for a definitive link between HPV and OSCC remains controversial.

The development of prophylactic HPV vaccines is predicted to have a major public health impact in the field of cervical cancer. If HPV is established as an aetiologic factor in OSCC, the prophylactic HPV vaccines may play an important part in reducing mortality from

*HS currently holds a National Health and Medical Research Council (NHMRC) Training Fellowship (1012631)—Australian Based Public Health Fellowship). ATN currently holds a National Health and Medical Research Council (NHMRC) Training Fellowship (630724—Australian Based Public Health Fellowship). ATN has previously received research funding for other projects from a manufacturer of HPV vaccine.
#Corresponding author.

OSCC, particularly in a country such as China where oesophageal cancer (OC) contributes significantly to the nation's cancer burden.

1.1. HPV

Papillomaviruses have non-capsulated icosahedral virions which are approximately 55nm in diameter and contain a genome of approximately 8000 base pairs [3]. The genome is surrounded by 72 capsomeres. The outer coat of the virus is comprised of a major and minor capsid protein. The HPV genome is comprised of three major regions and consists of circular double-stranded DNA which codes for 8 proteins. The Early region (E1-8) codes for genes associated with transcription, plasmid replication and transformation. The Late region consists of genes which code for the major (L1) and minor (L2) capsid proteins. The control region is responsible for producing the vital factors in the regulation of transcription and replication [4].

HPV infections have been linked to a broad range of mucocutaneous diseases, from benign skin warts to premalignant lesions and invasive carcinoma. Of the currently characterized HPV types, infection has been described in epithelial layers of the skin, the anogenital region and the oropharyngeal mucosa [5].

1.2. Human Papillomaviruses in Cancer

Currently, it has been estimated that HPV is responsible for 5.1 percent of the global cancer burden [6,7]. The mechanism of oncogenesis of HPV in cervical cancer has been well documented and may also be applicable to oesophageal mucosa if HPV is an aetiologic factor in OSCC. The integration of viral DNA into the host genome appears to be an important step in establishing the pathway to carcinogenesis [8]. Integration of HPV disrupts the viral E2 gene, which acts as a negative regulator of the E6/E7, the main viral genes responsible for immortalization and malignant transformation of the infected host cell. With loss of E2 control, unregulated expression of the E6 and E7 oncoproteins cause proteolytic degradation of the p53 and retinoblastoma (pRb) tumor suppressor genes respectively, effectively establishing malignancy [8,9].

To date, there has been no definitive description of how HPV could infect the oesophagus. However, as the oesophageal mucosa is continuous with that of the oropharynx, hypotheses related to transmission in HPV related oropharyngeal cancers have also been extended to OCs. Consequently, higher numbers of sexual partners, increasing practice of oral sex and initiation of sexual encounters at an earlier age have been associated with HPV-related oropharyngeal malignancies and could similarly be one of the risk factors for OCs in which HPV is isolated [10-13].

Some reports have also suggested a transplacental mode of transmission of HPV from infected mothers to their babies in utero as well as during passage of the infant through the birth canal. [14] This is supported by findings of genital tract HPV types 6, 11, 16 and 18 (usually found in the genital tract), in oesophageal tissue of newborns [15].

1.3. Oesophageal Squamous Cell Carcinoma

Of the main histologic subtypes of OC, OSCC accounts for the majority of oesophageal malignancies worldwide and is the predominant form of OC diagnosed in African and Asian countries [16,17].

The main aetiologic factors for OSCC are discussed below. The onset and progress of oesophageal cancer is insidious with few early symptoms, resulting in advanced disease at time of diagnosis for many patients. Endoscopy and barium swallow are the mainstay of OSCC diagnosis, with follow up endosonography and chest and abdominal computer tomography scans used for staging [18]. Dysphagia, odynophagia, dyspnoea, significant weight loss and other symptoms and clinical signs related to disseminated disease are generally reported and observed in patients with advanced OSCC. Prognosis is often poor and the five-year survival rate in most cases, is less than 10% [18].

1.4. Epidemiology of Oesophageal Cancer—How China Compares to the Rest of the Globe

OC is the eighth most common malignancy worldwide with an incidence of an estimated 500,000 new cases annually [6]. Approximately half of the world's OC cases occur in China where a reported annual incidence of 250,000 cases makes OC the nation's second most common malignancy, after lung cancer [19,20]. OC has a poor prognosis as it is usually diagnosed late, with a five year survival of less than 5% [21]. In 2008, oesophageal malignancy was responsible for 406,000 deaths globally, making it the sixth highest cause of cancer-related deaths [22] while in China, with an annual mortality of 150,000, it is the fourth leading cause of death from malignancy [23,24]. It is the second most common cause of cancer-related death in Chinese males [25]. **Figure 1** depicts the age-standardized mortality rates per 100,000 for oesophageal cancer in China, from 1990-1992. The most recent survey on oesophageal cancer incidence and mortality in China was carried out from 1998 to 2002 (**Table 1**). From the 30 cities and counties included, Ci County in Hebei Province had the highest incidence and mortality rates in age-standardized calculations for both men

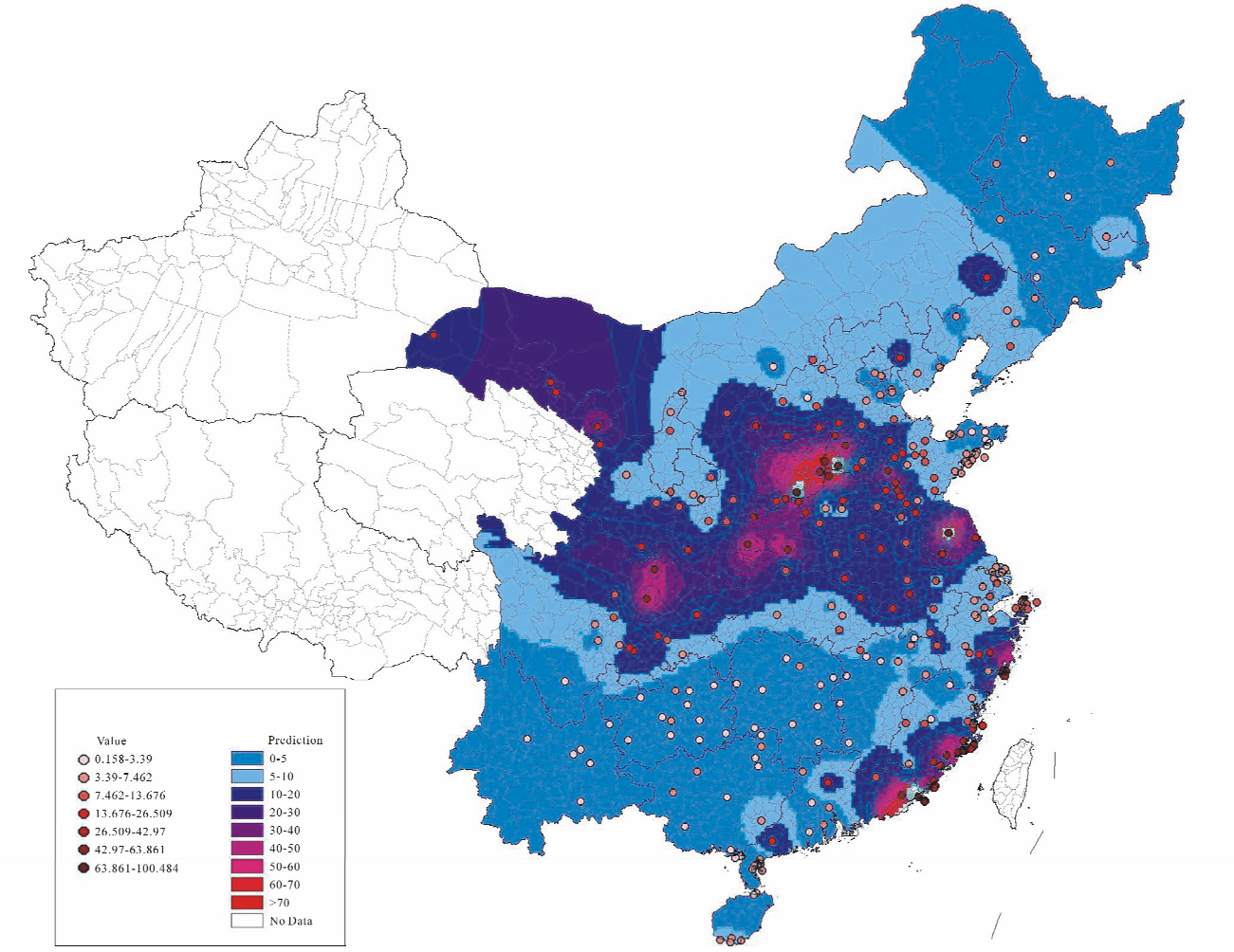

Figure 1. Estimated oesophageal cancer incidence per 100,000 in China, 1990-1992.

and women.

1.5. Epidemiology of OSCC in China

OSCC is predominantly a disease of developing nations and is the principal histologic type of OC in the Central Asian OC belt, which includes high-incidence countries such as China [26]. The average incidence rate for OC in the Chinese population is 13 per 100,000 [19] with OSCC representing more than 99% of all OC cases in China [27]. The variation in geographic incidence of OSCC internationally as well as within the same country, is well documented [28,29]. The major endemic regions within China are the northern Jiangsu province and the Linxian and Anyang counties in the eastern province of Henan [19,30], with mortality rates as high as 161/100,000 for males and 103/100,000 for females, in Linxian [31]. Significant differences in the incidence of OSCC also exist between regions of the same province in China, an intriguing and unexplained phenomenon. For instance, counties within the province of Hebei in the north of China, have reported incidence rates varying from 1.4 to 118.2 per 100,000 [32].

The broad range of incidence rates both regionally and globally has been ascribed to the complex, multifactorial aetiology of OSCC. In developed countries, tobacco use and excessive alcohol consumption are thought to be the two most important causative factors, responsible for 90% of OSCC cases [7,33-35]. However, in developing nations such as China, only a small percentage of OSCC cases can be attributed to alcohol and smoking [7,34,35]. In these high incidence areas, opium abuse, nutritional deficiencies [36,37], ingestion of hot food and beverages [38,39], exposure to nitrosamines, industrial chemicals, and certain viruses [40-45] such as HPV have also been implicated.

1.6. Evidence for Involvement of HPV in OSCC

In 1982, the carcinogenic potential of HPV in OC was

Table 1. Incidence and mortality rates for oesophageal cancer in China, 1998-2002[1] (data sourced from Li 2007) [47].

CITY/COUNTY	REGION	INCIDENCE (per 100,000)		MORTALITY (per 100,000)	
		Male	Female	Male	Female
Beijing	North	5.8	2.5	4.2	1.9
Tianjin	North East	5.5	2.2	4.6	1.9
Ci County, Hebei Province	North	122.3	77.8	107.8	57.2
Sha County, Fujian Province	South East	92.3	52.8	81.1	46.3
Yangcheng County, Shanxi Province	North East	119.7	69.3	95.4	49.6
Dalian, Liaoning Province	North	6.5	1.4	6.3	1.0
Anshan, Liaoning Province	North	6.7	1.7	5.3	1.6
Harbin, Heilongjiang Province	North	7.4	1.3	5.7	0.9
Shanghai	East	6.5	2.1	5.6	1.7
Haimen, Jiangsu Province	East	11.3	4.2	10.4	3.8
Qidong, Jiangsu Province	East	6.3	2.5	5.8	2.1
Huaian, Jiangsu Province	East	85.1	62.6	58.5	41.3
Yangzhong, Jiangsu Province	East	67.7	62.5	57.5	47.5
Hangzhou, Zhejiang Province	East	6.8	1.4	4.3	1.2
Jiaxing, Zhejiang Province	East	9.9	3.0	10.1	2.2
Jiashan County, Zhejiang Province	East	14.7	3.6	13.4	3.2
Haining, Zhejiang Province	East	11.4	3.1	9.7	2.8
Changle, Fujian Province	South East	12.9	4.1	11.0	3.5
Linqu County, Shandong Province	East	16.5	3.1	15.1	3.3
Feicheng, Shandong Province	East	61.7	27.8	55.1	25.3
Linzhou, Henan Province	East	74.5	51.8	63.7	37.2
Wuhan, Hubei Province	Central	8.6	2.7	7.1	2.1
Guangzhou, Guangdong Province	South East	7.0	1.4	6.0	0.9
Shenzhen, Guangdong Province	South East	38.7	21.1	6.6	3.1
Sihui, Guangdong Province	South East	6.0	1.7	6.7	1.8
Zhongshan, Guangdong Province	South East	12.6	1.4	9.5	1.0
Fusui County, Guangxi Province	South	3.3	0.7	3.6	0.6
Yanting County, Sichuan Province	West	99.5	58.9	83.4	48.7
Gejiu, Yunnan Province	South	0.5	0.0	NS	NS
Wuwei, Gansu Province	North West	53.4	21.8	NS	NS

NS—not specified.

first postulated by Syrjänen following the observation of HPV-related histological changes in OSCC tissue samples, identical to those seen in condylomas [46]. Subsequent investigations to assess a possible HPV-OSCC link, including experiments in animal models, serological, in vitro, and morphologic studies, have been well-documented [1,29]. Hypothesised modes of transmission of

[1]Rates are all age-standardized by Chinese standard population.

HPV in OSCC and mechanisms of oncogenesis based on a cervical cancer model, have also been previously summarized [28].

The most convincing studies have demonstrated the presence of HPV DNA sequences in OSCC tissue using techniques varying from Southern Blot to polymerase chain reaction (PCR), *in situ* hybridization (ISH) and immunohistochemistry (IHC). To date, the largest number of studies investigating the role of HPV in OSCC have been carried out in China, with some of these studies being published only in the Chinese language literature. To the best of our knowledge there have been no previous reports on this topic, which assess papers from both the English and Chinese language. We aim to review all studies conducted in China, in English and Chinese, with a view to informing prevention of OSCC in China through the use of prophylactic HPV vaccines, should an aetiological link to HPV be confirmed.

2. METHODS

2.1. Search Strategy

English and Chinese language papers included in this review were identified by searching the CNKI database as well as Medline, Embase and PubMed. Search terms included "human papillomavirus", "HPV", "oesophageal cancer", "squamous cell carcinoma" and "China". In addition, reference lists of retrieved articles were reviewed and hand searches of key journals including Annals of Oncology, Lancet Oncology, Anticancer Research, Gastroenterology, International Journal of Cancer, BMC Cancer, Diseases of the Esophagus, World Journal of Gastroenterology, Cancer Epidemiology Biomarkers & Prevention and Journal of Clinical Pathology, were conducted to source any articles which were not electronically indexed. There were no limitations to date of publication for either English or Chinese language studies and papers were sourced from the date when the databases started until February 2012.

2.2. Data Extraction

Articles met the selection criteria if they investigated the presence of HPV DNA in OSCC tissue in a Chinese cohort. All study types which included case series, cross-sectional and case-control studies, were accepted. Papers were searched and data were extracted by one author (SSL). All studies which met our search criteria were tabulated in chronological order (**Table 2**).

For each paper, data extraction included: 1) the year in which the study was conducted; 2) the geographic region of China from which subjects were recruited (**Tables 2** and **3**, **Figure 2**); 3) the testing methodology; 4) HPV types detected; 5) number of HPV positive OSCC samples compared to total number of OSCC samples tested; 6) if applicable, number of HPV positive controls compared to total number of control specimens tested; 7) the type of study; and 8) specimen retrieval method.

Recording of the specimen retrieval method is intended to assess whether HPV detection rates differ between deep and superficial OSCC test specimens, the sample retrieval method was recorded for all studies. Deep tissue was classified as surgical resections, diagnostic biopsies and formalin fixed and paraffin embedded samples; while superficial specimens included cell brushings and balloon cytology samples (**Table 2**).

The Chinese literature was also searched for the most recent epidemiological data on oesophageal cancer incidence and mortality and a summary of the results obtained from the source [47] are presented in **Table 1**. In addition, authors of this review, based at the Beijing Cancer Institute & Hospital Chinese Academy of Medical Sciences (CICAMS), generated a map (**Figure 1**) of oesophageal cancer mortality using data collected by CICAMS from 1990-1992, on 10% of the Chinese population. Based on OC mortality data collected for various counties in China, predicted mortality rates have been projected for surrounding regions (**Figure 1**).

2.3. Analysis of Case-Control and Cross-Sectional Studies

The case-control study design allows the investigator to estimate the odds of an outcome, such as OSCC, occurring when exposure to a potential risk factor such as HPV, has taken place. It is particularly useful as an initial study to determine causality, if a link between the exposure and outcome of interest, has not been previously established [48]. The case-control methodology is both time and cost-effective when investigating diseases with long latency periods, such as OSCC, because the disease state already exists at the start of the investigation [48]. Furthermore, case-control study design allows the simultaneous assessment of multiple risk factors, which is useful in diseases such as OSCC, which have a multifactorial aetiology [49]. Thus case-control studies are the most practical study design for examining the research question of whether HPV is an aetiological factor in OSCC.

This review defines cases as patients with OSCC and controls as healthy subjects from whom macroscopically normal oesophageal biopsy samples have been obtained. Papers which identify paraoesophageal tissue from OC patients, as controls, were not acknowledged in the control column of **Table 2** and were not classified as case-control studies in **Table 4**, as there is a significant possibility of cross-contamination and spread of HPV from the tumour into adjacent tissue, resulting in false-positive

Table 2. Identified studies of HPV in OSCC in China in English and Chinese language literature[2].

Year of publication	City/ province	Region of china	Detection method	HPV types detected	Method of oscc specimen retrieval	No. of hpv positive oscc samples/total tested (% hpv detection)	No. of positive control samples/total tested (% hpv detection)	Type of study	Ref
1989	henan	east	ihc	ag	ns	6/31 (19)	n/a	case series	[51]
1990	linxian, henan	east	hb	-	surgical specimen	25/51 (49)	n/a	case series	[102]
1990	linxian, henan	east	fish	11, 16, 18	surgical specimen	53/80 (66)	n/a	case series	[102]
1990	linxian, henan	east	ish	6, 11, 16, 18	surgical specimen	22/51 (43)	n/a	case series	[102]
1991[3]	chengdu, sichuan	south west	sb	16	surgical specimen	12/24 (50)	n/a	case series	[103]
1992	linxian, henan	east	pcr	6, 11, 16, 18	surgical specimen	25/51 (49)	n/a	case series	[15]
1992	linxian, henan	east	sb	11, 16, 18, 30	surgical specimen	8/20 (40)	n/a	case series	[15]
1993[3]	fujian	south east	pcr	cp	ffpe	24/40 (60)	n/a	case series	[56]
1993	linxian, henan	east	ish	6, 11, 16, 18, 30	surgical specimen	85/363 (23)	n/a	case series	[108]
1994	fuzhou, fujian	south east	pcr	6, 16	surgical specimen	24/40 (60)	n/a	case series	[57]
1995[3]	linxian, henan	east	sb, pcr	16, 18	surgical specimen	0/35 (0)	n/a	case series	[53]
1996[c]	zhengzhou, henan	east	ish	6, 11, 16, 18	ffpe	22/40 (55)	n/a	case series	[104]
1996	beijing	north	pcr	6, 16, 18	surgical specimen	3/70 (4)	n/a	case series	[77]
1996[c]	sichuan	south west	sb, pcr	16, 18	ffpe	37/103 (36)	n/a	case series	[60]
1997	sichuan	south west	pcr	16, 18	surgical specimen	32/152 (21)	n/a	case series	[25]
1997	ns	north	ish	wide spectrum	surgical specimen	3/36 (8)	n/a	case series	[105]
1999	anyang, henan	east	pcr	cp	diagnostic biopsies (3/70), surgical specimens (7/23), scrapings (10/24)	20/117 (17)	n/a	case series	[59]
1999	ns	ns	pcr	6, 9, 18, 20, 24, 51, 52, 57	surgical specimen	10/29 (34)	n/a	case series	[62]
2000[3]	shaanxi & gansu & sichuan & shanxi & hunan	central & north west & west & north east	pcr	11, 16	surgical specimen	ns/22 (ns)	n/a	case series	[95]
2000	linxian, henan	east	pcr	cp	surgical specimen	18/101 (17)	n/a	case series	[123]
2000	linxian, henan	east	ish	6, 11, 16, 18, 30, 53	surgical specimen	117/700 (17)	n/a	case series	[82]
2000[3]	shaanxi	central	ihc	e6	ffpe	44/60 (73)	24/56 (43)	case control	[101]
2000	beijing	north	pcr	cp	surgical specimen	ns/37 (ns)	n/a	case series	[94]
2001[3]	anyang, henan	east	is-pcr, ish	l1, e6, e7	surgical specimen	18/30 (60)	n/a	case series	[71]
2001	shangzhuang—anyang & tangmiao—neihuang	east	pcr, ish	16	ball cytology	2/2 (100)	50/112 (44)	cross sectional	[65]
2001	linxian, henan	east	pcr	cp	ball cytology	2/32 (6)	4/57 (7)	case control	[72]
2002	anyang, henan	east	pcr, ish	16, 18	ball cytology	39/62 (63)	17/36 (47)	case control	[64]
2002	eastern guandong	south east	pcr	cp	surgical specimen	115/176 (66)	n/a	case series	[73]

Continued

2003[3]	ns	ns	pcr	cp	surgical specimen	28/40 (70)	n/a	case series	[78]
2003[3]	cixian, hebei	north	pcr, ihc	cp	surgical specimen	28/152 (18)	n/a	case series	[68]
2003	anyang & shanxi	east & north east	pcr,ihc,ish	16	surgical specimen	31/48 (65)	n/a	case series	[80]
2003	hong kong & sichuan & linxian & shantou & xi'an	south & south west & east & south east & central	pcr	16, 18	surgical specimen	43/319 (13)	n/a	case series	[76]
2004	hong kong & sichuan & linxian	south & west & east	pcr	16, 18	surgical specimen	18/87 (21)	n/a	case series	[75]
2004	xinjiang	north west	pcr	16	surgical specimen	55/104 (53)	n/a	case series	[69]
2004[3]	beijing	north	ihc	e6, e7	ffpe	15/18, 16/18 (83, 89)	n/a	case series	[100]
2004[3]	guandong	south east	ihc, pcr	16	ffpe	14/30 (47)	7/60 (12)	case control	[83]
2005[3]	anyang & beijing	east & north	ish	16	ffpe	86/119 (72)	n/a	case series	[106]
2005	anyang, henan	east	pcr	16, 18	surgical specimen	207/265 (78)	203/357 (57)	case control	[55]
2005[3]	jiangsu	east	pcr, hb	ns	cell brushing	23/60 (38)	11/60 (18)	case control	[85]
2006	shantou	south east	ish	16, 18	surgical specimen	24/60 (40)	n/a	case series	[111]
2006	henan & hubei	east & central	ish, ihc	16, 18	surgical specimen	ns/82 (ns)	ns	unk	[107]
2006	linxian, henan	east	ish	-	ball cytology	0/4 (0)	61/475 (13)	cross sectional	[52]
2007[3]	shanghai	east	ish	16	surgical specimen	59/90	2/20	case control	[110]
2007[3]	xinjiang	north west	pcr	18, 31, 45	ffpe	ns/316 (ns)	n/a	case series	[96]
2007	anyang, henan	east	pcr	16	surgical specimen	97/161 (60)	n/a	case series	[81]
2007[3]	sichuan	south west	pcr	16	surgical specimen	43/112 (38)	n/a	case series	[67]
2007	anyang, henan	east	pcr	16, 31, 51, 56, 53, 73	surgical specimen	11/100 (11)	n/a	case series	[58]
2007	gansu & shandong	north west & east	pcr, sb	16, 18	surgical specimen	19/59 (32)	n/a	case series	[74]
2007[3]	anyang & zhengzhou, henan	east	pcr, hb	16	surgical specimen	54/110 (49)	7/45 (16)	case control	[87]
2008[3]	linzhou, henan	east	pcr	16, 18	surgical specimen	29/31 (94)	n/a	case series	[63]
2008	xinjiang	north west	pcr	6, 11, 16, 18, 31, 52, 66	surgical specimen	20/67 (30)	n/a	case series	[70]
2008[3]	xinjiang	north west	pcr	16	ffpe	58/150 (39)	4/40 (10)	case control	[84]
2008[3]	chongqing	central	pcr	16, 18	surgical specimen	43/112 (38)	n/a	case series	[88]
2009[3]	guandong & henan	south east & east	pcr	16, 18, 45, 33, 58, 59, 73, 31, 56	surgical specimen	0/140 (0)	n/a	case series	[54]
2009[3]	henan	east	pcr	16	surgical specimen	37/44 (84)	n/a	case series	[97]
2009[3]	hebei	north	pcr	16, 18	surgical specimen	37/42 (88)	n/a	case series	[79]
2009[3]	xinjiang	north west	pcr	16	ffpe	23/63 (37)	21/126 (17)	case control	[66]
2009[3]	xinjiang	north west	pcr	16, e6	surgical specimen	26/82 (32)	n/a	case series	[91]
2010	shantou	south east	pcr	16, 18, 58	surgical specimen	35/70 (50)	20/60 (33)	case control	[99]

Continued

2010	henan	east	pcr	16	surgical specimen	8/17 (47)	n/a	case series	[98]
2010	xinjiang & anyang & shantou	north west & east & south east	pcr	6, 16, 18, 26, 45, 56, 57, 58	ns	160/347 (55)	n/a	case series	[124]
2010	linxian, henan	east	pcr	89, 16, 31	surgical specimen	3/267 (1)	n/a	case series	[61]
2010	shaanxi	central	pcr	16	surgical specimen	35/69 (51)	2/32 (6)	case control	[89]
2010[c]	xinjiang	north west	pcr	16, e6	ffpe (biopsy)	46/100 (46)	22/100 (22)	case control	[86]
2011	changhua, taiwan	south east	pcr, ish	6, 11, 16, 18	surgical specimen	2/31 (6)	n/a	case series	[93]
2011[c]	jiangsu	east	ish	16, 18	ffpe	40/72 (56)	7/48 (15)	case control	[109]
2011[c]	linzhou, henan	east	pcr	16	diagnostic biopsy	18/18 (100)	n/a	case series	[92]

Ag—HPV Antigens; CP—consensus primers; GP—general primers; HB—histological biopsy; IHC—immunohistochemistry; ISH—*in situ* hybridization; PCR—Polymerase chain reaction; SB—southern blot hybridization; FFPE—formalin fixed and paraffin embedded; N/A—Not applicable as study did not include controls; ND—Not Determined; UNK—Unknown due to insufficient information for determining study type; NS—not specified.

Figure 2. Representation of the number of HPV-OSCC studies carried out in various provinces in China.

HPV detection rates. We identified 14 case control studies and a further 2 cross-sectional studies (**Table 4**) investigating OSCC tissue compared to oesophageal tissue from healthy controls, for the presence of HPV DNA in

[2]Control subjects in this paper are defined as individuals who do not have a diagnosis of OSCC. Therefore studies which have determined controls to be biopsies of macroscopically normal oesophageal tissue adjacent to the primary tumour, have been discounted;
[3]Chinese language papers. The remaining papers are in English.

Table 3. HPV Detection Rate by Region in China, based on 64 identified studies from the English and Chinese Literature[d].

REGION	NO OF OSCC POSITIVE SAMPLES/TOTAL TESTED (% HPV DETECTION)
North	154/447 (34)
North West	228/566 (40)
South East	300/643 (47)
South West	124/391 (32)
South	16/123 (13)
East	1173/3415 (34)
Central	132/298 (44)
Unspecified Regions	38/69 (55)
China (Total)	2165/5952 (36)

the Chinese population. Epi Info™ 3.5.3 [50] was used to calculate odds ratios (OR) with 95% confidence intervals, for the association of HPV with OSCC, by cross-tabulating the summary data presented in the papers for case-control and cross-sectional studies. P-values for the significance of the ORs were calculated from chi-squared test. Only two of the identified studies presented calculations of ORs. Few studies adjusted for confounding factors and our calculation of unadjusted odds ratios for the association of HPV with OSCC from the summary data provided in the papers, must therefore be interpreted in this context.

3. Results

The first study looking for an aetiological link between HPV and OSCC in China was carried out in 1989 using immunohistochemistry (IHC) [51]. In total, 64 studies

Table 4. Case-Control and Cross-Sectional Studies Examining HPV DNA in OSCC in China, from English and Chinese language literature[4].

YEAR	REGION	METHOD	HPV TYPES FOUND	POSITIVE NUMBER CASES (%)	POSITIVE NUMBER CONTROLS (%)	CRUDE ODDS RATIO[5]	P-VALUE	REF
2000	Shaanxi (Central)	IHC	E6	44/60 (73)	24/56 (43)	3.67 (1.57 - 8.65)	0.0009	[101]
2001	Shangzhuang - Anyang & Tangmiao - Neihuang (East)	PCR, ISH	16	2/2 (100)	50/112 (44)	Incalculable	0.1192	[65]
2001	Linxian, Henan (East)	PCR	CP	2/32 (6)	4/57 (7)	0.88 (0.1 - 6.13)	0.8898	[72]
2002	Anyang, Henan (East)	PCR, ISH	16,18	39/62 (63)	17/36 (47)	1.90 (0.76 - 4.75)	0.1305	[64]
2004	Guandong (SE)	IHC, PCR	16	14/30 (47)	7/60 (12)	6.63 (2.04 - 22.23)	0.0002	[83]
2005	Anyang, Henan (East)	PCR	16,18	207/265 (78)	203/357 (57)	2.71 (1.86 - 3.94)	<0.0001	[55]
2005	Jiangsu (East)	PCR, HB	NS	23/60 (38)	11/60 (18)	2.77 (1.12 - 6.97)	0.0151	[85]
2006	Linxian, Henan (East)	ISH	Nil	0/4 (0)	61/475 (13)	0.00 (0 - 10.68)	0.4429	[52]
2007	Anyang & Zhengzhou, Henan (East)	PCR, HB	16	54/110 (49)	7/45 (16)	5.23 (2.02 - 14.12)	0.0001	[87]
2007	Shanghai (East)	ISH	16	59/90 (66)	2/20 (10)	17.13 (3.46 - 114.6)	<0.0001	[110]
2008	Xinjiang (NW)	PCR	16	58/150 (39)	4/40 (10)	5.67 (1.8 - 19.87)	0.0006	[84]
2009[6]	Xinjiang (NW)	PCR	16	23/63 (37)	21/126 (17)	2.88 (1.36 - 6.11)	0.0023	[66]
2010	Shaanxi (Central)	PCR	16	35/69 (51)	2/32 (6)	15.44 (3.20 - 101.46)	<0.0001	[89]
2010	Shantou (SE)	PCR	16,18,58	35/70 (50)	20/60 (33)	2.00 (0.92 - 4.35)	0.0552	[99]
2010[7]	Xinjiang (NW)	PCR	16, E6	46/100 (46)	22/100 (22)	3.02 (1.56 - 5.86)	0.0003	[86]
2011	Jiangsu (East)	ISH	16,18	40/72 (56)	7/48 (15)	7.32 (2.69 - 20.71)	<0.0001	[109]
	Total			681/1239 (55)	478/1684(28)			

CP—consensus primers; HCII—Hybrid Capture 2; IHC—immunohistochemistry; ISH—*in situ* hybridization; LR—Low-risk HPV types; HR—High risk HPV types; PCR—Polymerase chain reaction.

[4]Table excludes studies which did not report the number of HPV positive OSCC samples from the total tested.
[5]Unadjusted ORs calculated using Epi Info™ 3.5.3, from summary data presented in the papers. As adjustments for confounding factors have not been carried out in most studies, it is important to interpret these ORs with caution. Only two studies calculated ORs as highlighted below.
[6]OR calculated by authors of study: 2.67 (1.38 - 5.17); $P < 0.05$.
[7]OR calculated by authors of study: 3.020; $P < 0.001$.

have been conducted in China to date, which include a total of 6409 OSCC samples as summarised in **Table 2**. Of these, 36 were in the English language and 29 were in Chinese. Of the 64 studies investigating the role of HPV in OSCC in China, a majority of 47 were case series, with 14 case-control studies, 2 cross-sectional studies and 1 report of indeterminable study design (**Table 2**). From all studies conducted in the Chinese population, 2166/5953 (36%) of all OSCC tissue and 478/1684 (28%) of all healthy control tissue, tested positive for HPV (**Table 2**).

3.1. Regions of China

To date, the Chinese population has contributed the largest number of OSCC specimens for HPV analysis, compared to any other country [28]. Henan province in East China is the site of over 50% of all national studies (**Figure 2**). Of all OSCC samples tested in this high incidence area, 34% yielded positive HPV results (**Table 3**). Other high-incidence areas within China from the north-west, south-east, and the central regions have reported even higher rates of HPV DNA detection in OSCC tissue, ranging from 40% - 47% (**Table 3**).

3.2. Testing Methods

As summarized in **Table 2**, a variety of techniques have been used in these studies to detect HPV. The detection rate of HPV in OSCC tissue varied from 0 to 100%. Only three studies did not detect HPV in any of the OSCC samples tested [52-54]. Forty-nine of 64 studies utilized PCR with HPV detection rates varying widely from 0% - 100% [15,25,53-99]. PCR yielded a HPV positive rate in 37% of all OSCC tissue tested using this method. Approximately one quarter of all OSCC specimens were analysed by ISH, with HPV detected in 30% of these samples. Six studies have used IHC to test OSCC tissues samples with HPV detection rates varying from 18% - 89% [51,68,80,83,100,101]. One study reported a 66% HPV detection rate using filter *in situ* hybridisation (FISH) [102], five studies used southern blot hybridization [15, 53,60,74,103] and a further three studies examined histological biopsies of oesophageal lesions [85,87,102].

Sixteen studies used ISH with percentage of OSCC tissues testing positive for HPV, varying from 0% - 72% [52,64,65,71,80,82,93,102,104-111]. ISH was the methodology used in the largest study to be carried out on this topic, analyzing a total of 700 OSCC samples from the Henan Province, with an HPV detection rate of 17% [82]. High-risk HPV types 16 and 18 were the most commonly detected genotypes in this study, as well as in all other investigations from the Chinese cohort.

3.3. Site of Specimen Retrieval

We also examined studies for any potential relationship between HPV detection rates and whether OSCC test specimens were superficial cell scrapings or deep tissue biopsies. Of the 60 studies which had sufficient information for analysis, one tested both superficial and deep oesophageal tissue specimens, 54 tested only deep tissue and the remaining 5 tested superficial specimens (**Table 2**). We found that 76/184 (41%) of superficial OSCC samples and 1990/5607 (35%) of deep OSCC tissue samples tested positive for HPV DNA.

3.4. Results of Case-Control and Cross-Sectional Studies

As highlighted in **Table 4**, we identified 14 case-control studies and 2 cross-sectional studies which investigate the association of HPV DNA in OSCC tissue compared to normal control tissue in the Chinese population. From these studies, 681/1239 (55%) of all OSCC samples and 478/1684 (28%) of all healthy control tissue tested positive for HPV. Only two of these studies calculated an odds ratio in the published papers [66,86]. Table 4 presents these results as well as our unadjusted, crude odds ratios which were calculated using Epi Info™ 3.5.3 (CDC) [50]. Our analysis demonstrates that 11/16 studies had statistically significant odds ratios which support a potential HPV-OSCC link. The largest study, carried out in the high incidence County of Anyang in Henan Province [55], reported 207/265 (78%) OSCC tissues testing positive for HPV DNA against 203/357 (57%) controls and had an unadjusted odds ratio of 2.71 (p-value < 0.0001).

4. Discussion

Our findings of a statistically significant association of HPV with OSCC in 11/16 case-control and cross-sectional Chinese studies, suggest that HPV may be a potential aetiological factor in OSCC, in the Chinese population. However, as our unadjusted ORs have been calculated from summary data in papers, many of which have not adjusted for important confounding factors, it is important to interpret our results accordingly.

In the 16 studies in China which have tested control subjects for HPV [52,55,64-66,72,83-87,89,99,101,109, 110], a number of controls have tested positive for HPV, including two studies in which HPV was isolated from a greater percentage of controls than cases [52,72]. Interestingly, both of these studies were carried out in Linxian within Henan province, a region which has one of the highest incidence rates of OC in the world [30]. Fidalgo *et al.* also reported 100% of controls testing positive for HPV DNA in a Portuguese cohort [112]. This trend may be suggestive of an early role for HPV in the aetiology of

OSCC, in which normal oesophageal mucosa infected by HPV may undergo malignant transformation following expression of the E6/E7 viral oncogenes [77].

We found that HPV types 16 and 18 are the most commonly detected genotypes within oesophageal tissue in China. However, it is also important to note that a majority of studies to date test only for the main oncogenic genotypes of HPV, namely types 16 and 18, thereby raising the possibility that less common HPV types are missed in the testing process. Nevertheless, some HPV types, which have not previously been isolated from oesophageal tissue, have been described in some studies which have tested for a broader range of HPV types. Of note are types 30, 53, 56, 66, 73 and 89 in the Chinese cohort (**Table 2**). In particular, Chang *et al.*, reported 8 out of 85 HPV-positive OSCC samples with HPV 30 [108]. As HPV 30 has only previously been identified in two genital condylomas [113], and one malignant laryngeal lesion, the finding of this HPV genotype in eight OSCC samples has led to proposals that HPV 30 may have a proclivity to infect oesophageal mucosa [108].

This review underscores the highly variable results of HPV detection in OSCC, between different regions of China, for which diverse testing methodology may be a contributing factor. It is difficult to draw conclusions on which testing methods yield the highest and lowest rates of HPV detection since certain techniques such as FISH, SB and HB have been employed in very few studies and PCR which has been used in 76% of studies has also shown variable results between studies.

While general trends have reported higher HPV DNA detection rates in OSCC tissue from high incidence OSCC regions, the results of our review did not demonstrate this pattern. Thirty-four percent of all OSCC samples, sourced predominantly from the highest OSCC incidence region of Henan Province in eastern China, were positive for HPV DNA. However, we found that other high-incidence areas within China from the north-western counties in Xinjiang, to Guandong in the south-east, Hebei in the north and the central Province of Shaanxi have reported even higher rates of HPV DNA detection in OSCC tissue. Of note is a study carried out by Koshiol where only 3/267 OSCC samples tested using PCR, were positive for HPV [61]. This is one of the largest studies to be carried out and their result is particularly interesting as it has been conducted in Linxian, Henan province [30]. This result is not in keeping with the generally observed trends of high HPV detection rates in high-risk OSCC populations. Furthermore, the three studies in China which did not isolate HPV from OSCC, also recruited subjects residing in Henan province [52-54].

A recent study by Furrer *et al.* reported higher rates of HPV DNA detection in superficial oral scrapes compared to deep tissue biopsies from patients with oral cancer, in an Argentinian cohort [114]. They suggest that the site of specimen sampling is important in obtaining an accurate epidemiological picture on the HPV link to carcinogenesis. This hypothesis may be extended to oesophageal cancer and we therefore aimed to examine whether the location from which the oesophageal specimen was taken in OSCC patients, *i.e.* superficial scrapings or deep tissue biopsy, may have any correlation with HPV detection rate. We found that 35% of deep tissue biopsies and 41% of superficial scrapings from OSCC patients were HPV positive. This finding is consistent with results reported by Furrer *et al.*

With the growing evidence that the spectrum of HPV-related malignancies may spread beyond cancers of the anogenital tract [1], the global health burden attributable to HPV continues to increase. As a result, there has been increasing pressure to make the HPV vaccines more widely available for males, thereby immunizing entire cohorts against the effects of this virus. If HPV plays a significant role in the aetiology of OSCC, the introduction of prophylactic HPV vaccines could have a public health impact in a nation such as China where OC is one of the leading causes of malignancy-related mortality.

In many developed countries public health funding for the prophylactic HPV vaccines is available for girls and young women prior to their sexual debut [115]. However, in China, there are currently no national programs for cervical cancer screening and a majority of women have never been screened. Consequently, at present, the prophylactic HPV vaccines have not been licensed for distribution in China.

Population-based surveys of Chinese women have been carried out recently to identify potential difficulties in the implementation of a prophylactic vaccination program in China [116,117]. One of the largest obstacles is the price of the vaccination, should government funding be insufficient to cover costs [116,117]. Prophylactic HPV vaccines are to date the most expensive vaccine developed with a retail price of US $120 per dose of Gardasil® (US $360 for the complete course) excluding administrative costs [118]. Other problems include rural habitation with poor access to health services, cultural and religious barriers, personal attitudes and beliefs as well as limited knowledge of HPV and vaccination [116, 117]. However, the introduction of the Hepatitis B vaccine (HBV) to protect against hepatocellular carcinoma (HCC) in China may provide a template upon which the prophylactic HPV vaccination program can be modeled [117]. The first national HBV program was instigated in Taiwan in 1984 [119]. Over a 10 year period from 1984 to 1994, follow-up studies in children under the age of 15, demonstrated a reduction in HBsAg prevalence rates

from 9·8% to 1.3% [120]. A recent cross-sectional seroprevalence study by Chang *et al.* also reported a statistically significant reduction in incidence of HCC in a cohort of vaccinated children aged 6-19 years, compared to a comparable unvaccinated group [119].

From the results of this review, we determine that HPV DNA was found in over one third of OSCC tissue samples, compared to cervical cancer where HPV is responsible for the pathogenesis of 100% of lesions. Thus the impact of prophylactic vaccination would be considerably higher in cervical cancers than in OSCC, if a link exists. However, it is important to clarify any HPV-OSCC association as even a 20% - 30% rate of HPV infection as a causal co-factor would be significantly impacted by vaccination, particularly in a geographically targeted vaccination program.

5. Conclusions

We found that 36% of all OSCC samples and 28% of all healthy control samples tested from the Chinese population were positive for HPV DNA and the majority (11/16) of case-control and cross-sectional studies found a statistically significant association between HPV and OSCC.

The findings of this review are in line with the hypothesis that HPV detection rates are higher in superficial oesophageal cancer samples compared deep tissue specimens [114]. It may therefore be important to consider depth of tissue biopsy when interpreting epidemiological studies assessing HPV aetiology in malignancy.

Research carried out over the last 30 years has neither precluded nor established HPV as an aetiological factor in OSCC. The difficulty in determining a link may be due to several factors including 1) the poor methodological design and generally smaller sample sizes in a majority of studies. Only few case-control studies have ever been done, with the vast majority of studies on the subject being case-series, which are unable to adequately address the question of aetiology or risk factors. The fact that none of the identified case-control studies included statistical measures of association, even when data were collected to enable these measures, indicates the problem of poor study design; 2) the utilization of many different HPV detection methods with varying specificity and sensitivity ranges *i.e.* PCR with either general or consensus primers which identify different HPV genotypes, histological biopsy (HB), IHC, ISH, FISH, general primer (GP), consensus primer (CP), serological testing, hybrid capture; 3) inter-laboratory deviation on similar testing methodology; 4) utilization of various types of specimens *i.e.* balloon cell samples, OSCC tissue from resections or biopsies which may be either fresh or archival; 5) variation within tissue samples examined; 6) differences in histopathological classification and tissue storage *i.e.* Iodine staining, paraffin samples; 7) the presence of potential co-factors (e.g. smoking, opium abuse, nutritional deficiencies, ingestion of nitrosamines and exposure to other industrial chemicals) which may be more important depending on geographical location, could act synergistically with HPV to promote infection of oesophageal tissue; 8) the possible "hit and run" mechanism proposed by Campo and modeled on observations of bovine papillomavirus type 4 (BPV-4) infection of bovine oesophageal tissue [121]; 9) genetic polymorphisms facilitating malignant transformation [122].

Despite the many factors which could be responsible for the high variability of results reported, it remains that an equal potential for inconsistency with similar variables existed in investigations carried out to establish the role of HPV in cervical and other HPV-related cancers, which have yielded more convincing results. Therefore, it may be inferred that if a link does exist between HPV and OSCC, it may be weaker than in other HPV-related cancer, or geographically varied and related to other co-factors [52].

A meta-analysis of existing case-control studies as well as further large-scale case-control studies with adequate statistical power are required to more meaningfully address whether a causal relationship between HPV and OSCC exists. The introduction of the prophylactic HPV vaccines has made it even more important to definitively determine the answer to this research question, particularly for countries such as China, where there is a significant cancer burden from oesophageal malignancy.

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Abbreviations

BPV: Bovine papillomavirus
CICAMS: Cancer Institute & Hospital Chinese Academy of Medical Sciences
CDC: Centre for Disease Control
CNKI: Chinese National Knowledge Infrastructure
CP: Consensus primer
FISH: Filter *in situ* hybridisation
GP: General primer
HCC: Hepatocellular carcinoma
HB: Histological biopsy
HPV: Human papillomavirus
IHC: Immunohistochemistry
ISH: *In situ* hybridisation
IARC: International Agency on Research on Cancer
OC: Oesophageal cancer
OSCC: Oesophageal squamous cell carcinoma
PCR: Polymerase chain reaction

