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Prevalence of human papillomavirus and its prognostic value in vulvar cancer: A systematic review and meta-analysis

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Abstract

The purpose of this study was to estimate the prevalence of human papillomavirus (HPV) in vulvar cancer and determine whether positive HPV in vulvar cancer was associated with a better prognosis. Literature searches of Ovid EMBASE, PubMed, Web of Science and Cochrane Library were performed to identify related studies published from January 2000 to May 2017. A total of 33 studies including 7,721 subjects were selected in this meta-analysis. Overall, the HPV prevalence in vulvar cancer tissue was 34% (95% CI: 28%-39%) with 45% (95% CI: 28%-64%) in Asian populations and 34% (95% CI: 26%-42%) in Caucasian populations. The HPV-positive vulvar cancer was associated with better overall survival (hazard ratio = 0.64, 95% CI: 0.47–0.87; $P = 0.004$) and recurrence-free survival (hazard ratio = 0.66, 95% CI: 0.45–0.97; $P = 0.03$) compared with HPV-negative counterpart. HPV status may play an important role in predicting the prognosis of patients with vulvar cancer. The HPV-positive vulvar cancer women might relatively have a better survival than HPV-negative ones.

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Introduction

Vulvar cancer is the fourth most common type of gynecological cancer and encompasses approximately 6% of all female genital tract malignancies. According to cancer statistics, there have been more than 6,000 cases and 1,150 deaths every year in the United States [1]. Although the incidence of vulvar cancer is low, it has increased over the past few decades, particularly amongst younger women [2–6]. Although patients with early stage vulvar cancer have a favorable prognosis, the patients with advanced disease have poor treatment outcomes [7]. Therefore, it is important to improve the prognosis of vulvar cancer, especially for patients with advanced stage of vulvar cancer.

Ninety percent of vulvar cancers are squamous cell carcinomas [8, 9] and several other morphological variants mainly include basaloid, keratinising, warty and verrucous carcinoma [10]. One third of vulvar cancer cases are basaloid and warty variants, which are more common in younger women and are often associated with human papillomavirus (HPV) infection.

On the contrary, keratinising variants caused by chronic vulvar dermatosis are not associated with HPV and predominantly occur in older women [11].

There are more than 100 types of HPV, and these are divided into 3 broad categories according to their oncogenic potential [12–14]. The association between HPV infection and some types of gynecological tumors have been identified in cervical, endometrial, and ovarian cancers. However, due to the low frequency of vulvar cancer, only a few detailed studies were focused on the effect of HPV infection on the survival outcomes in vulvar cancer patients. Therefore, the prognostic significance of HPV infection in vulvar cancer has not been fully understood yet and is still debatable. Some studies reported that patients with HPV-positive tumors have a better prognosis than those with HPV-negative tumors, whereas others did not reach the same conclusion. The HPV prevalence in vulvar cancer cases ranges in different studies from 3.3% to 76.5%; the inconsistent results of the prognostic value of HPV in vulvar cancer may be due to the diverging prevalence rates of HPV.

To our knowledge, the prevalence and prognostic value of HPV infection in vulvar cancer reported in previous studies have not been subjected to statistical pooling. In order to validate these personal observations, the aims of this study were to evaluate the HPV prevalence, determine the prognostic value of HPV and clarify which variables may be the underlying causes of heterogeneity in prevalence and prognostic value of HPV in vulvar cancer.

Methods

Data sources and search strategy

Literature searches of Ovid EMBASE databases, PubMed, Web of Science, and Cochrane Library databases were performed from January 2000 to May 5, 2017. The main keywords used for the search were "carcinoma or cancer or malignancy or adenocarcinoma or neoplasm or carcinoma" and "vulva or vulvar or genito-urinary or genitourinary or genital or genitalia or perineum or perineal" and "papilloma virus or papillomavirus or papillomavirus infections or HPV". The detailed search terms and strategies are shown in S1 Table. The articles published were limited to English language. Additionally, the citation lists of retrieved articles were manually screened independently by two authors. All selected studies were checked according to a Newcastle-Ottawa Quality assessment Scale developed previously [15].

Selection criteria

Inclusion criteria of this meta-analysis were as follows: (1) independently published study, investigating the prevalence of HPV in vulvar cancer patients; (2) a study investigated the association between HPV status and survival outcomes in vulvar cancer patients. The following exclusion criteria were also applied: (1) reviews; (2) case report or case series; (3) studies lacking enough information.

Data extraction and quality assessment

Two investigators (Jianxin Zhang and Yang Zhang) independently performed the data extraction and quality assessment. The detailed information collected for each study mainly included first author, publication year, ethnicity, country, study period, types of vulvar cancer, type of tissue, HPV prevalence, HPV Types and detection methods, survival indicators, HR estimates, and follow-up time., The studies were merged into a unique extraction, if several publications were overlapped. If a study hadn't reported the HR and its related 95%CI, Kaplan-Meier survival curves could be used referring to previously published methods [16, 17]. Additionally, the discrepancies were resolved via discussion.

The quality of each eligible study was assessed by the nine-star Newcastle–Ottawa Scale (NOS). A study would be considered to have high quality with the NOS score equal or greater than seven scores. After data extraction and assessment, the information would be examined and adjudicated independently by an investigator (Zhenyu Zhang).

Statistical analysis

Of the studies identified, the overall prevalence of HPV in vulvar cancer was analyzed, using the R software (version 3.4.1) and was estimated based on a random-effects model, in which the between-study variance was determined with the Der-Simonian Laird estimator. The results of the overall prevalence of HPV in vulvar cancer for all studies sorted by first author were presented using forest plots. The prevalence of HPV among vulvar cancer patients from each study was presented with exact binomial 95% confidence intervals (CIs).

The meta-analysis of the prognostic value of HPV in vulvar cancer was carried out using Review Manager (RevMan) 5.3 analysis software (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). The association between HPV and survival outcomes in patients with vulvar cancer was estimated by calculating pooled HRs and related 95% CI. If the eligible articles did not report the HRs and 95% CI, they would be extracted according to previously published methods. The results were presented using forest plots. The study heterogeneity was assessed and presented by Chi^2 and I^2. I^2 values of 25, 50 and 75% were defined as low, moderate, and high estimates, respectively. If no study heterogeneity existed ($P>0.10$ or $I^2<50\%$), the meta-analysis would use the fixed-effects model; otherwise, use the random-effects model. A HR<1 indicated a better survival outcome for HPV-positive while HR>1 indicated a worse survival outcome for HPV-positive. A P value less than 0.05 was considered statistically different. The sensitivity analysis was performed by deleting each study in turn to assess the consistency and quality of the results. The funnel plot and Egger's test were performed to assess the potential publication bias.

Results

Literature search and study selection

The process of literature search and study selection is summarized in Fig 1. A total of 9,284 studies were collected using the detailed search strategies in the 4 databases selected. After reading the titles and abstracts, 47 potential studies were included for full-text view. With further screening, 33 studies, [18–50] reporting the HPV prevalence in vulvar cancer were identified according to the inclusion criteria. The main characteristics of the studies included are summarized in Table 1. There were 5 studies reported on Asian populations and 23 studies on Caucasian populations. The types of HPV in these studies mainly included HPV 6, 11, 16, 18, 31, 33. In addition, the majority of these eligible studies used the formalin-fixed paraffin-embedded tissue to perform the HPV detection and genotyping. The majority of these eligible studies used the PCR-based methods for the detection of HPV.

Of the 33 studies, 9 studies reported the association between HPV infection and survival outcomes among vulvar cancer patients. The main characteristics of the studies included are summarized in Table 2. The survival indicators mainly included the overall survival (OS, n = 8), progression-free survival (PFS, n = 1), recurrence-free survival (RFS, n = 2), disease-free survival (DFS, n = 2), and disease-specific survival (DSS, n = 2). The HR estimates methods included HR combined with 95% CI and calculated according to the Kaplan-Meier survival curves.

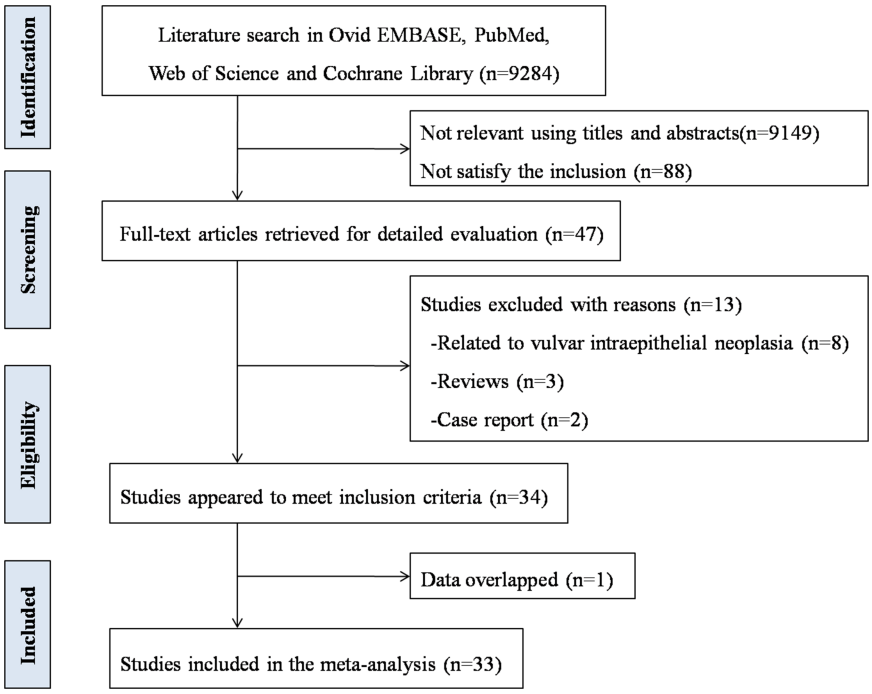

Fig 1. Flow chart of the selection process for the eligible studies.

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HPV prevalence in vulvar cancer

A forest plot of the 33 eligible studies is shown in Fig 2. The forest plot showed the pooled prevalence of HPV for all studies with the prevalence of HPV in vulvar cancer for each study. The prevalence of HPV in vulvar cancer varied from 3.3% to as high as 76.5%. From the results of the meta-analysis, the pooled prevalence of HPV in vulvar cancer was 33.7% (95% CI: 28.5%-39.4%).

The subgroup analysis was performed depending on the ethnicity (Fig 3). The pooled prevalence of HPV in Asian populations was 45% (95% CI: 28%-64%), whereas it was 34% (95% CI: 26%–42%) in Caucasian populations. There was no significant difference in HPV prevalence between the two populations ($P = 0.2582$).

HPV status and survival outcomes in vulvar cancer

Nine studies provided data on the association between HPV status and survival outcomes in vulvar cancer patients. The pooled analysis showed that the HPV-positive vulvar cancer was associated with better OS (HR = 0.64, 95% CI: 0.47–0.87; $P = 0.004$; Fig 4) and RFS (HR = 0.66, 95% CI: 0.45–0.97; $P = 0.03$; Fig 5) compared with their HPV-negative counterparts. In addition, HPV-positivity tended to be associated with better DFS (HR = 0.90, 95% CI: 0.54–1.48; $P = 0.66$; Fig 6), and DSS (HR = 0.15, 95% CI: 0.00–9.89; $P = 0.38$; Fig 7).

Correlation between HPV prevalence and the OS in vulvar cancer

To investigate the correlation between HPV prevalence and the OS in vulvar cancer for the 8 studies, we had plotted the prevalence and the OS (with 95%CI bars) for the 8 studies in a 2 dimension graph (Fig 8). The results ($r^2 = 0.4360$, $P = 0.0747$) showed that there wasn't a

Table 1. The main characteristics of the 33 studies included in the meta-analysis.

Study	Ethnicity	Country	Study period	Study types	Cancer Types	Tissue Types	Quality assessment of samples	HPV Prevalence		HPV Types	Detection Methods
								n/N	%		
Alonso, et al (2011)	Caucasians	Spain	1995–2009	Hospital-based	VSCC	FFPE	β-globin PCR analysis	19/98	19.4%	16, 31, 33, 51, 52, 56	SPF-10 primers, INNO-LiPA HPV Genotyping kit
Antonets, et al (2013)	Caucasians	Russian	NA	NA	VC	FFPE	NA	12/58	20.6%	16, 18, 31, 33, 35, 45, 51, 52, 58, 59	PCR
Engelman, et al (2003)	Mixed	Brasil	1983–1995	Institution-based	IVSCC	FFPE	NA	4/55	7.3%	16/18	ISH
Felez-Sanchez, et al (2016)	Caucasians	Spain	NA	Institution-based	VSCC	FFPE	Tubulin PCR analysis	30/902	3.3%	2, 16, 33, 45, 52, 53, 54, 66, 70, 74	PCR-SPF10/DEIA/LiPA25
Fuste, et al (2010)	Caucasians	Spain	1990–2007	Institution-based	VSCC	NA	NA	18/94	19%	16	PCR
Gargano, et al (2012)	Caucasians	United States	1995–2005	Registry-based	IVC	FFPE	β-globin PCR analysis	121/176	68.8%	16,18,31,33,45,52,59	PCR-PGMY9/11 primers and type-specific hybridization; retesting with SPF10
Hampl, et al (2008)	Caucasians	Germany	1980–2007	Institution-based	VC	NA	NA	18/36	50%	6, 11, 16, 18, 33, 42, 52, 55	PCR
Huang, et al (2005)	Asians	China	NA	Institution-based	VSCC	Frozen	β-globin PCR analysis	6/8	75%	16, 18	PCR
Karnezis, et al (2015)	Caucasians	Canada	1985–2005	Hospital-based	VSCC	FFPE	NA	77/193	40%	NA	NA
Kim, et al (2015)	Asians	Korea	1998–2011	Institution-based	VC	FFPE/Frozen	NA	15/35	42.86%	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	HC2 test
Kowalewska, et al (2010)	Caucasians	Poland	2003–2006	Institution-based	VSCC	FFPE	β-globin PCR analysis	7/46	15%	6, 16, 58	Linear Array HPV Detection Kit
Koyamatsu, et al (2003)	Asians	Japan	1982–1998	Institution-based	VC	FFPE	NA	4/31	12.8%	16,18	PCR
Larsson, et al (2012)	Caucasians	Sweden	1983–2008	Hospital-based	VSCC	FFPE	HMBS PCR analysis	40/130	30.8%	6, 11, 16, 18, 31, 33, 39, 45, 51, 52, 56, 58, 59	PCR
Lee, et al (2016)	Caucasians	United States	1985–2011	Institution based	VSCC	FFPE	β-globin PCR analysis	15/56	27%	16, 18, 27, 33	multiplex PCR
Lindell, et al (2010)	Caucasians	Sweden	2000–2007	Institution based	VSCC	FFPE	Housekeeping gene by PCR	23/75	31%	6, 11, 16, 18, 33	PCR(GP5+/6+ and CPI/IIG)
Menczer, et al (2000)	Caucasians	Israeli	NA	NA	VC	FFPE	NA	9/14	64.2%	16, 18	PCR, HPV negative cases were re-examined with a sensitive primer.
Ngamkham, et al (2016)	Asians	Thailand	2003–2012	Institution based	VC	FFPE	β-globin PCR analysis	16/34	47.1%	6, 11, 16, 18, 31, 33, 35, 45, 58	PCR-EIA
Ordi, et al (2016)	Caucasians	Spain	1980–2011	NA	VSCC	FFPE	NA	184/791	23.3%	NA	SPF10PCR/DEIA/LiPA25 system
Pinto, et al (2004)	Mixed	Brazil	1975–1992	Hospital-based	VC	FFPE	β-globin PCR analysis	38/161	23.6%	6, 11, 16, 18, 45	PCR and DBH (GP5+/GP6+)
Poblet, et al (2010)	Caucasians	Spain	NA	Hospital-based	VC	FFPE	NA	11/37	30.3%	16, 18, 33, 35	PCR(GP5+/GP6+ and My09/My11)

(*Continued*)

Table 1. (Continued)

Study	Ethnicity	Country	Study period	Study types	Cancer Types	Tissue Types	Quality assessment of samples	HPV Prevalence		HPV Types	Detection Methods
								n/N	%		
Rakislova, et al (2016)	Caucasians	Spain	NA	NA	VSCC	NA	NA	452/ 1636	27.6%	NA	NA
Reuschenbach, et al (2013)	Caucasians	Germany	2003–2009	Institution based	VC	FFPE	NA	80/ 183	43.7%	6, 11, 16, 18, 26, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82	PCR, a multiplex test based on the Luminex technology
Rodrigues, et al (2013)	Mixed	Brazil	1979–2006	Institution-based	VSCC	FFPE	β-globin PCR analysis	34/ 87	39.1%	16, 18, 31, 33, 35, 42, 45, 53,54, 71, 82, 84	Linear array HPV- test
Rumbold, et al (2012)	Australia	Australia	2007–2009	Institution based	VC	Fresh	β-globin PCR analysis	201/ 521	38.6%	6, 11, 16, 18	PCR PGMY09/ 11, Roche Linear Array
Sagdeo, et al (2014)	Caucasians	United States	NA	Institution based	VSCC	FFPE	β-globin PCR analysis	13/ 17	76.5%	16, 18, 33, 45, 53, 120	PCR (PGMY-GP +-primer system)
Santos, et al (2006)	Caucasians	Spain	1995–2005	Hospital-based	VSCC	FFPE	β-globin PCR analysis	16/ 92	17.4%	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, 82	PCR(GP5+/GP6 +, SPF10)
Serrano, et al (2015)	Mixed	48 countries	NA	Hospital-based	VC	FFPE	NA	489/ 1709	28.6%	6, 11, 16, 18, 31, 33, 45, 52, 58	SPF-10PCR/ DEIA/ LiPA25 System
Siriaunkgul, et al (2014)	Asians	Thailand	2006–2012	Hospital-based	VSCC	FFPE	β-globin PCR analysis	29/ 47	62%	16, 26, 58, 89	PCR (MY09/11 and GP5+/GP6 +.), Linear Array Genotyping Test
Sutton, et al (2008)	Caucasians	United States	1987–2007	Institution-based	VSCC	FFPE	β-globin PCR analysis	81/ 116	69.8%	6, 16, 18, 26, 33,45, 52, 61	PCR-Linear Array HPV Test
Sznurkowski, et al (2016)	Caucasians	Poland	2002–2007	Institution-based	VC	FFPE	RNAseP gene PCR analysis	38/ 85	45%	16, 18, 33, 39, 59	SPF10–LiPA25 system
Tsimplaki, et al (2012)	Caucasians	Greece	NA	Hospital-based	VSCC	FFPE	β-globin PCR analysis	3/6	50%	16, 18, 31, 33, 45	PapilloCheck DNA Microarray
Van, et al (2009)	Caucasians	The Netherlands	1988–2005	Institution-based	VSCC	FFPE	β-globin PCR analysis	45/ 130	34.6%	16, 18, 33, 52, 53,54, 58, 66	PCR and ISH
Wakeham, et al (2017)	Caucasians	UK	2001–2014	Institution-based	VSCC	FFPE	β-globin PCR analysis	32/ 62	52%	6, 11, 16, 18, 33, 51, 53	Optiplex HPV Genotyping assay

DEIA: DNA enzyme immunoassay; EIA: Enzyme-immunoassay; FFPE: Formalin-fixed paraffin-embedded; ISH: in situ hybridization; IVC: invasive vulvar cancer; IVSCC: invasive squamous cell carcinoma; NA: not available; VC: vulvar cancer; VSCC: vulvar squamous cell carcinoma

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significant correlation between the prevalence and the OS in vulvar cancer for the 8 studies in our meta-analysis.

Qualitative assessment

Quality assessment of the 33 eligible studies is shown in S2 Table. The average NOS score of the eligible studies was 7.03 (ranged from 6 to 8), which indicated that the majority of the eligible studies were high quality.

Sensitivity analysis

A sensitivity analysis was performed for assessing the results of this meta-analysis (data not shown). Results of the sensitivity analysis showed that no significant alteration of the pooled

Table 2. The main survival indicators of the 9 studies for the meta-analysis.

Study	Survival Indicators	HR type	HR Estimates	Follow-up
Alonso, et al (2011)	OS, DFS	Age-adjusted	HR and 95%CI	3.8 years (range: 0.9 to 5 years)
Kim, et al (2015)	OS, DFS	Age-adjusted	KM	2.8 years (range:0.3 to 18.9 years)
Larsson, et al (2012)	OS	No-adjusted	HR and 95%CI	NA
Lindell, et al (2010)	OS, RFS, DSS	Age and tumor size -adjusted	KM	42.0 months
Pinto, et al (2004)	OS, RFS	Age-adjusted	HR and 95%CI	59.9 months (rang: 1 to 265 months)
Rodrigues, et al (2013)	OS	No-adjusted	KM	5 years
Sznurkowski, et al (2016)	OS	No-adjusted	KM	89.20 months (range: 1.7–189.5 months)
Van, et al (2009)	DSS	No-adjusted	KM	Last: August 1, 2008
Wakeham, et al (2017)	OS, PFS	Age and cancer stage adjusted	HR and 95%CI	5.8years (range 55 days to 14 years)

NA: not available; KM: Kaplan-Meier; OS: Overall survival; PFS: Progression-Free Survival; RFS: Recurrence-Free Survival; DFS: Disease-Free Survival; DSS: Disease-Specific Survival; HR: harzard ratio.

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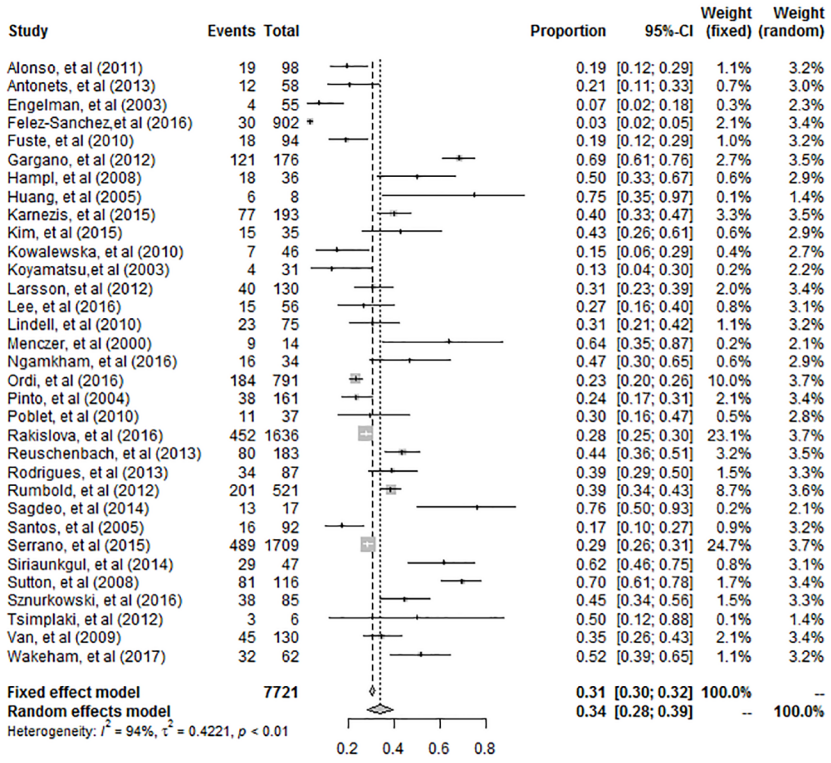

Fig 2. Forest plot of the prevalence of human papillomavirus in vulvar cancer.

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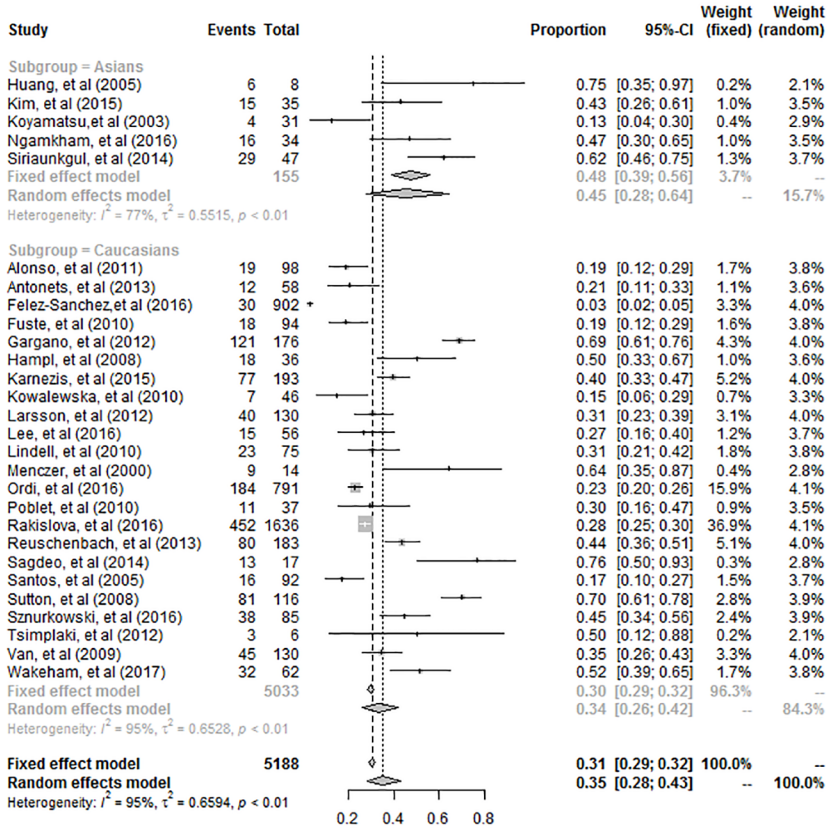

Fig 3. Forest plot for the subgroup analysis of ethnicity.

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incidence and HRs existed after removing a single study one by one, which indicated that the results of the prevalence and prognostic value of HPV in vulvar cancer were relatively stable and reliable.

Publication bias

The publication bias in the eligible studies, reporting survival outcomes of vulvar cancer was assessed by the funnel plot and Egger's test. The shape of the funnel plot was approximately symmetrical (Fig 9). Additionally, the Egger's test suggested that no publication bias was existed (P = 0.487).

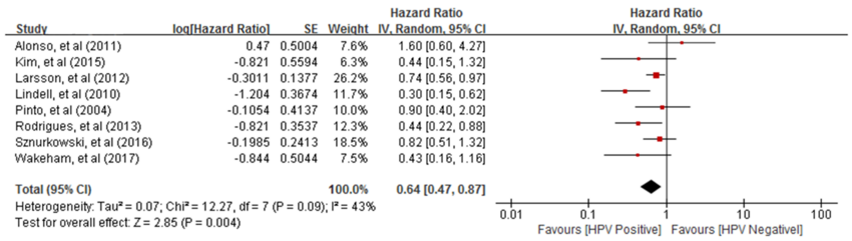

Fig 4. Forest plot for the association between human papillomavirus and overall survival in patients with vulvar cancer.

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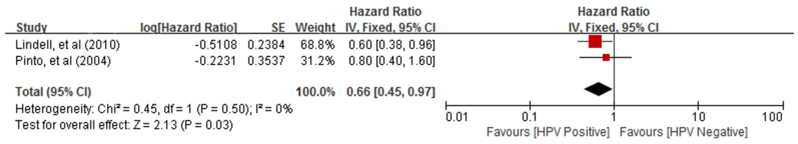

Fig 5. Forest plot for the association between human papillomavirus and recurrence-free survival in patients with vulvar cancer.

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Discussion

Vulvar cancer is a rare type of gynecological cancer among women worldwide and is more commonly diagnosed in older women (over 65 years) (5–7, 23). Based on the discovery and identification of prognostic biomarkers, the therapeutic approaches of vulvar cancer will evolve from standard radical resections to more individualized approaches (35, 36). Because the definition of new prognostic variables might result in further individualization of the treatment of vulvar cancer, it would be useful to confirm more prognostic biomarkers of vulvar cancer to guide the individualized treatment.

HPV was found to have a causal role in some types of gynecological cancers such as cervical cancer [51]. It was reported that persistent high-risk HPV infection was the essential cause for the development of vulvar abnormal lesions or progression of vulvar cancer [52]. Furthermore, several studies found the prognostic significance of HPV infection for vulvar cancer; however, with controversial results. Meanwhile, the prevalence rates of HPV were also inconsistent in the reported studies. Although a previous systematic review and meta-analysis had estimated the pooled prevalence of HPV in vulvar cancer, this study ignored the prognostic value of HPV in vulvar cancer (53). Thus, we conducted a systematic review and meta-analysis to investigate the prevalence rates of HPV and to clarify the real association between the HPV status and survival outcomes in patients with vulvar cancer.

In this meta-analysis containing 33 studies and 7,721 cases of vulvar cancer, the pooled prevalence rate of HPV was 34% (95% CI: 28%–39%) with large study heterogeneity (I^2 = 94%, P<0.01). The greater diverging prevalence rates of HPV positivity in these studies could generally be explained by different HPV detection methods (PCR, ISH, etc.), different case selection, or focusing on different types of HPV. In contrast to the established role of HPV as a risk factor, a significantly better survival outcome for women with HPV-positive tumors was found, compared to women with HPV-negative tumors. The pooled HRs of the associations between HPV status and OS, RFS indicated risk factors of 0.64 and 0.66 with significant P values, respectively. In addition, HPV-positive status tended to be associated with better DFS and DSS, although there was no statistical difference. This lack of significance could be partially explained by the small number of articles, as only two studies were included.

The drivers as to why HPV-associated vulvar cancer is associated with improved outcomes compared to their HPV negative counterparts are not fully established. Some factors, such as age at diagnosis, tumor size, subtype and clinical stage, morphology and histopathologic grade

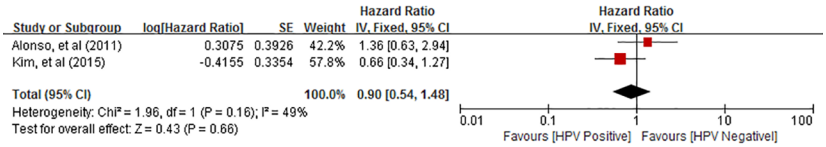

Fig 6. Forest plot for the association between human papillomavirus and disease-free survival in patients with vulvar cancer.

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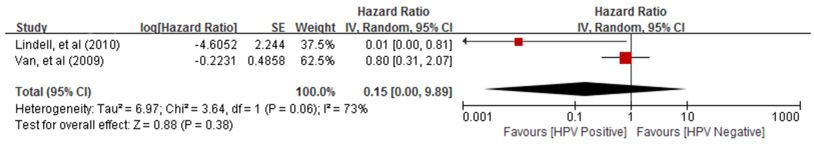

Fig 7. Forest plot for the association between human papillomavirus and disease-specific survival in patients with vulvar cancer.

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are important to predict tumor prognosis as well. However, we could not analyze the impact of each factor on the prognostic value of HPV in vulvar cancer patients because of the limitation of data. We could not determine whether the HPV status is an independent prognostic factor for vulvar cancer. Some studies found that patients with HPV-positive tumors had better survival, when adjusted for age and tumor size, than patients with HPV-negative tumors [32]. However, it was also reported that HPV-positive cases showed better OS than those of HPV-negative ones, while multivariate analysis did not show an independent prognostic significance [40, 50]. As the HRs in the majority of the eligible studies were age-adjusted, our results might be with a relative higher credibility indicating HPV-positive vulvar cancer women might relatively have a better survival than HPV-negative ones. However, it needs more evidence to support this conclusion.

Vulvar cancer develops through 2 distinct molecular pathways, one involving high-risk HPV infection and often observed in the younger patients less than 50 years old, and the other through early p53 suppressor gene mutation and often observed in the elderly patients. We speculate that the different pathogenesis and characteristics of the two types of vulvar cancer may partially explain the different survival outcomes. Notably HPV-positive (and p16-positive) patients were reported to be less likely to have recurrence and there were no vulvar cancer related deaths, whereas p53-mutant positive patients had a greater probability of recurrence

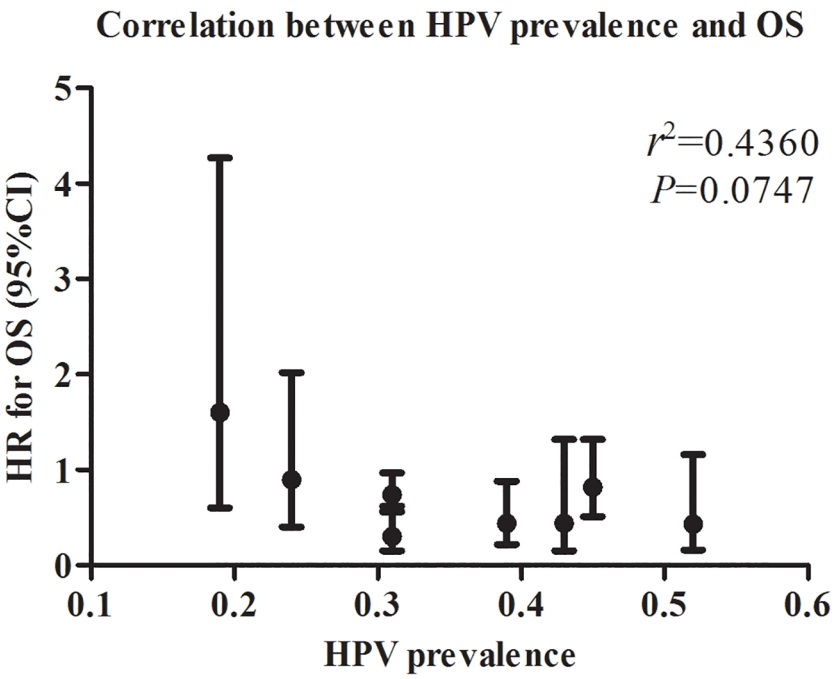

Fig 8. Correlation between HPV prevalence and the OS in vulvar cancer for the 8 studies.

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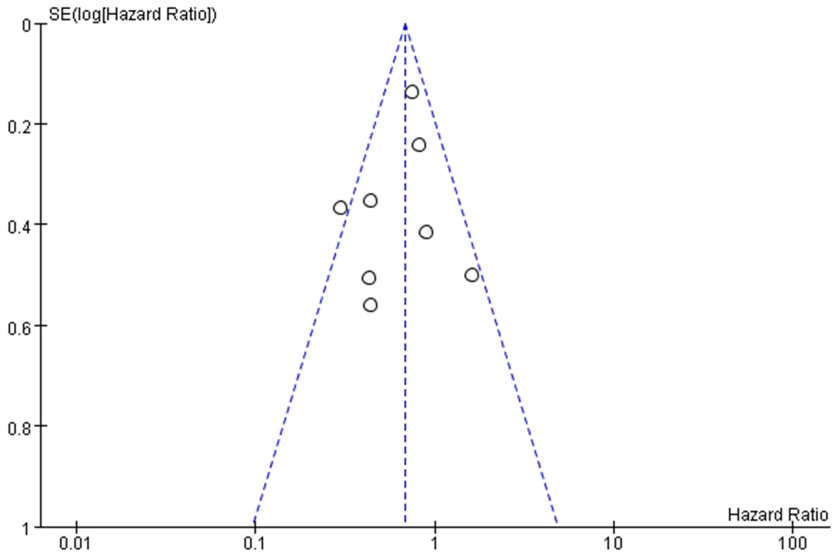

Fig 9. Funnel plot of prognostic value of human papillomavirus in vulvar cancer for publication bias.

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and were significantly more likely to die from vulvar cancer [53]. Moreover, HPV-positivity was more common in younger patients, while HPV-negativity was more common in elder patients of vulvar cancer. Age was proved to be an effective prognostic indicator in vulvar cancer [10, 27]. Furthermore, other factors associated with HPV status could also influence the prognosis of vulvar cancer patients. For example, a recent study conducted by Rodrigues, et al. indicated that loss of β-catenin and high Slug, Snail and Twist expression was associated with HPV-negative tumors [40]. The alterations in β-catenin and Slug expression may increase the risk of deeper invasion and metastasis characteristic due to a potentially more aggressive behavior of the tumor cells in the tumor front. Because of the lack of EMT-like events, the patients with HPV-positive tumors may usually have better prognosis. Meanwhile, HPV-negative tumors, which develop EMT-like events, would increase capability of invasion and progression, therefore leading to worse prognosis and poorer outcomes.

There are some limitations of this meta-analysis. Firstly, some relevant studies were excluded in the meta-analysis due to incomplete raw data or publication limitations. Secondly, studies in other databases might have been lost. Thirdly, among the studies that reported the association between the HPV infection and survival outcomes in vulvar cancer, several studies that needed to calculate HR and its related 95% CI might have caused potential bias and imprecise values. Additionally, not all of the primary studies included in our meta-analysis analyzed the other many important prognostic factors comprehensively. Therefore, we could not determine whether HPV status is an independent prognostic factor for vulvar cancer. Furthermore, due to the large numbers of variables such as HPV types, detection methods and so on in studies about HPV prevalence and the limited studies about the prognostic value of HPV on vulvar cancer, we couldn't determine the underlying causes of heterogeneity in prevalence and prognostic value.

In conclusion, our study is the first meta-analysis to explore the prognostic value of HPV infection in vulvar cancer. We demonstrated a high prevalence of HPV-positivity in vulvar cancer cases which was similar with the previous study (53). The HPV status may act as a biomarker for predicting the prognosis of patients with vulvar cancer. More large-scale and well-

designed studies are needed to confirm whether HPV status is an independent prognostic factor for vulvar cancer in the future.

Supporting information

S1 Table. The search strategy for the prevalence and prognostic value of human papillomavirus in vulvar cancer.
(DOC)

S2 Table. Application of the quality assessment tool NOS to the studies included in the meta-analysis.
(XLSX)

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