Degree project

UNIVERSITY OF SKÖVDE

THE SEVERITY OF HUMAN PAPILLOMAVIRUS- 16/18 INFECTION AND ITS PREVENTION TO CERCIVAL CANCER: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Abstract

Infectious diseases caused by human papillomavirus (HPV) are among the most common sexually transmitted diseases in the world. Currently, all countries of the WHO Eastern Mediterranean Region (EMRO) except the United Arab Emirates and Libya do not have a national vaccination program including the HPV vaccine. Cervical cancer risk can be reduced through the use of prophylactic HPV vaccines. Hence, the aim of this study was to examine the severity of HPV-16/18 infection in cervical cancer through a systematic review and to evaluate the effectiveness of vaccines against HPV-16/18 variants to prevent cervical cancer via a meta-analysis. Both the systematic review and meta-analysis contain nine relevant studies with 66154 and 78308 cervical cancer participants respectively. Statistical analyses were performed using pooled odds ratios (OR) with 95% confidence intervals (95% CI). Publication bias was examined using the funnel plot graph. The findings stated that overall 70% of cervical cancer was attributed to either HPV 16 or HPV 18. Heterogeneity for this meta-analysis was found to be I^2= 80% with a *p-value* <0.01 and overall OR (odds ratio) was 0.09 (95% CI= 0.04-0.20) for the random effect model. The lower odds ratio (less than 1) indicated fewer occurrences of cervical cancer in the HPV 16/18 vaccinated group than in the unvaccinated individuals. The overall vaccination efficiency was found to be 91% from the odds ratio ((1-0.09)x100=91). Thus, the present findings support that a prophylactic vaccine against HPV16/18 prevents the severity of HPV-associated cervical cancer.

Popular Scientific Summary

Persistent infection with high-risk HPV is the main risk factor for invasive cervical carcinoma. The HPV virus is the most common sexually transmitted infection (STI) and has been linked to 15 different types of cancer. Among the HPV diseases, cervical cancer accounts for the majority of deaths in resource-limited nations. Since the 1970s, cervical cancer mortality rates have decreased with improved medical care and nationwide screening programs. In many developing nations, knowledge, and practices relating to health are inadequate, making awareness and vaccination programs essential. HPV vaccines and routine cervical screening are the best ways to reduce the incidence of cervical cancer.

The present study focuses on evaluating the effectiveness of vaccines on HPV-16/18 variants against cervical cancer. A systematic review was conducted by collecting nine relevant articles to examine the severity level of HPV 16/18 strains causing cervical cancer. The findings stated that overall 70% of invasive cervical cancer were attributed to either HPV 16 or HPV 18. Other 18% of invasive cervical cancer were associated with the types of HPV (31, 33, 35, 45, 52, and 58). Then a meta-analysis was conducted to assess the efficiency of the HPV vaccination program to prevent the risk of cervical cancer. A total of 95 papers were identified, out of which nine relevant randomized clinical trials were selected. The nine studies were randomized multicentered phase III and II controlled clinical trials.

Overall, 78308 women of ages 15-26 years old (39124 CIN cases and 39184 control cases) from the selected trials were comprised in this meta-analysis of HPV 16/18 vaccine efficacy. Statistical analyses were performed using mean differences or pooled odds ratios (OR) with 95% confidence intervals (95% CI). Using OR, the prediction of the severity level of cervical cancer among the HPV-vaccinated group can be measured. The higher odds ratio (greater than 1) indicates a larger number of occurrences of cervical cancer after the vaccination in the treatment group. If the odds ratio is less than 1, cervical cancer is less likely to occur in the treatment group. The overall OR for this study was found to be 0.09 (95% CI= 0.04-0.20) for the random effect model. The cumulative vaccine efficacy was found to be 91%. This indicates that, after treatment, vaccinated individuals had significantly fewer levels of cervical cancer than unvaccinated individuals. A significant reduction rate of cervical cancer recurrence and prevention of HPV infection was observed in the vaccinated group when compared with the control non-vaccinated group. Heterogeneity for this meta-analysis was found to be I^2= 80% by a *p-value* <0.01. Generally, heterogeneity in meta-analyses refers to the difference in outcomes between studies. Here, the meta-analysis showed that differences in the vaccination sources, publication year and participant number may be the factor responsible for heterogeneity.

Based on the results, the vaccine was effective, well tolerated, and immunogenic in a broad range of young and adult women. This supports its potential use to prevent CIN and cervical cancer. It would be possible to significantly reduce the global burden of cervical cancer through vaccination in young women and low-cost HPV DNA screening in older women, but such an approach would require a demonstration project before widespread adoption could occur. The benefits of the vaccine must be optimized for each individual and for the entire population over time. In the future, cancer immunoprevention through vaccination will be a standard part of long-term cancer treatment.

Keywords: HPV-16/18 infection, Cervical Cancer, Therapeutic vaccine, Meta-analysis, systemic review

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Abbreviations

APC	:	Antigen-presenting cells
AS04	:	Adjuvant System 04
CI	:	confidence intervals
CIN	:	Cervical intraepithelial neoplasia
CTL	:	Cytotoxic T lymphocytes
EMRO	:	Eastern Mediterranean Region
FDA	:	Food and Drug Administration
HPV	:	Human papillomavirus
ICC	:	Invasive cervical cancer
IFN-1	:	Interferons Type-1
LSILs	:	Low-grade squamous intraepithelial lesion
OR	:	Odds Ratios
Pap test	:	Papanicolaou test
PCR	:	Polymerase chain reaction
RCT	:	Randomized control trial
SIL	:	Squamous intraepithelial lesions
STI	:	Sexually transmitted infection
Th-1 or 2	:	T helper cells 1 or 2
TLR-4	:	Toll-like receptor 4
TNF-α	:	Tumor necrosis factor-α

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1. Introduction

Cervical cancer is the third most cause of women's cancer death worldwide, with nearly 530000 new cases and 275000 deaths per year (Bradford & Goodman, 2013). Nearly 70% of cervical cancer cases are due to the persistent infection of oncogenic HPV-16/18 variants (Kovacevic et al., 2019). Most people do not know about they have been infected with the HPV virus and therefore may inadvertently transmit the HPV infection to their partners (Mays et al., 2004). HPV types associated with cervical cancer have been assessed in a previous meta-analysis including approximately 10000 cases. The eight most common HPV variants analyzed for cervical cancer are HPV 16, 18, 45, 31, 33, 52, 58, and 35, which account for 90% of all cervical cancers worldwide (Ciapponi, et al. 2011).

Despite the increased production of HPV vaccines and screening strategies, cervical cancer remains the most common gynaecological cancer worldwide (Pimple et al., 2016). There are three different types of HPV vaccines are available (bivalent, quadrivalent, and nonavalent) against high-risk HPV 16/18 (Van de Velde et al., 2012). These HPV vaccines are Gardasil 9, 9vHPV, quadrivalent HPV vaccine Gardasil, 4vHPV, and bivalent HPV vaccine Cervarix, 2vHPV respectively (Lin et al., 2020). These vaccines enhance the synthesis of antibodies against HPV-like particles. The large incidence and mortality of cervical cancer are due to poor healthcare infrastructure, lack of effective screening and early detection programs, and cancer treatment facilities (Denny et al., 2006). Vaccination against HPV is an effective and simple method to prevent cervical cancer. Cytology-based screening of HPV reduced the incidence of cervical cancer (Basu et al., 2018).

This study focused to assess the proportion of cervical cancer attributable to high-risk HPV 16/18 types covered by the prophylactic HPV vaccines, through a systematic review and a meta-analysis. Meta-analysis is based on systematic statistical analysis that combines the results of multiple scientific studies, which aims to develop a quantitative estimate of the studied phenomenon.

1.1 Human papillomavirus -16/18 infection

Human papillomaviruses (HPV) belong to the Papillomaviridae family of small double-stranded circular DNA viruses (Figure 1.). HPV infection is the most common sexually transmitted infection (Gibson et al., 2014). Most of the infected person does not show any symptoms but can pass the infection to others through close skin-to-skin contact (Risser et al., 2005). Nearly 150 different types of HPV have been identified, with different outer capsid proteins L1. The HPV viral capsid contains 72 capsomeres which are made up of L1 and L2 capsid proteins (Belnap et al., 1996). HPV infects the basal cells of the squamous epithelium via microinjury. High-risk HPV strains differ from other strains by the oncogenic efficiency of E6 and E7 proteins which leads to uncontrolled cell growth and genome mutation (Faridi et al., 2011). HPV E6 and E7 proteins are mainly expressed in basal cells of the squamous epithelium and induce epithelial cell proliferation. HPV E1 and E2 proteins are expressed in the middle epithelial layers and carried out viral DNA replication (Faridi et al., 2011). The HPV E4 protein is expressed in the middle to upper epithelial cell layers and alters the keratin network of the host cell cytoplasm for the propagation of viral components. HPV proteins E6 and E7 inhibit the expression of type-1 interferons (IFN-1), retinoblastoma protein and *p53* in host cells (Yang et al., 2017). Thus, the downregulation of inflammatory signals in the host cells may induce immune tolerance. In addition, the E5 protein suppresses the expression of HLA-class 1 and it facilitates the evasion of cytotoxic T lymphocytes attack (Seliger et al., 2006). Hence, the immune evasion mechanism gradually supports the spread of persistent HPV infection leading to the development of cervical cancer. HPV mainly replicates on the infected basal cells of stratified epithelium. Cutaneous infection (skin wart) and mucosal infection both are caused due to HPV strains. HPV 16 is the most virulent compared to HPV 18 type. These two types are associated with approximately 70% of all cervical cancers and also cause anal, vaginal, vulvar, and penile cancers. HPV infections do

not show any symptoms in the initial stage, the infected persons are thus not aware of being infected hence leading to rapid spreading.

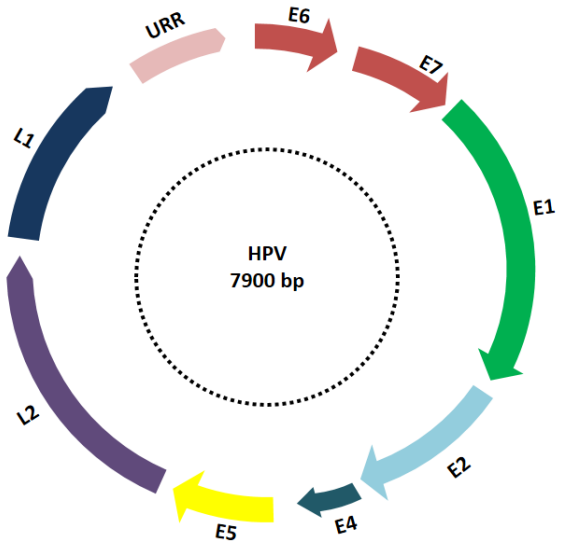

Figure 1. HPV virus genome: The human papillomavirus genome is a circular DNA structure containing approximately 7900 base pairs that encode several genes. E1 and E2 are viral replication proteins. E4 and E5 are used for the genome amplification process. Both E6 and E7 produce oncogenic proteins. Capsid proteins L1 and L2 make up the viral envelope. URR stands for the upstream regulatory region.

1.2 The role of innate immunity in HPV infection

In general, the invasion of viruses into the body will tend to release interferons for the inhibition of viral replication and secretion of immune cells. This immune response is non-specific to all types of antigens and is termed innate immunity. In HPV-infected conditions, E6 and E7 proteins have been shown to suppress the transcription of interferons in host cells. Hence, HPV 16/18 infection downregulates the innate immune mechanism via the inhibition of interferons (Stanley, 2012). Macrophages and natural killer cells also play an important role in the innate immune system. When macrophages bind to the viral genome through toll-like receptors, they will secrete inflammatory cytokines and interferons and kills virally infected cells via the secretion of tumor necrosis factor (TNF-α). In the presence of TNF- α, monocyte chemotactic protein -1 which is released by normal keratinocytes, attracts more macrophages into the site of viral infection, whereas such secretion is inhibited by the E6 and E7 proteins (Stanley, 2012). This leads to the downregulation of macrophage infiltration into the HPV-infected area.

1.3 The role of adaptive immunity in HPV infection

After the HPV infection antigen-presenting cells (APC) capture the viral proteins and lysis them into peptide fragments. These cells then move to the secondary lymphoid organ where the viral peptide is now presented by MHC-2 cells. Hence, HPV infection is recognized by this immune response and adaptive immunity is activated (Didierlaurent et al., 2009). Various inflammatory cytokines such as IL-1, IL-6, IL-12 and TNF-α are secreted by the activated immune cells for inducing inflammation, which is significant for the aberrant activation of the adaptive immune system. The antigen-presenting signal is recognized by CD4+ T lymphocytes and they differentiated into T helper cells (Th-1 and Th-2 lymphocytes) (Didierlaurent et al., 2009). Th-1 lymphocytes enhance the cell-mediated immune responses and Th-2 lymphocytes activate the B-cells for antibody secretion. The cytokines secreted by the Th-1 lymphocytes activate the cytotoxic T lymphocytes (CTL) for the production of effector T cells which can kill cancer-inducing

HPV infected host cells. Activated CTL for the production of effector T cells which can kill cancer inducing HPV antigens infected host cells (Sasagawa et al., 2012).

1.4 Malignant transformation

HPV E6 and E7 proteins are efficiently blocking the maturation of HPV infected host cells by negatively regulating the cell cycle checkpoints (Boulet et al., 2007). These genomic alterations will eventually drive the malignant transformation of HPV infected cells into invasive cancer celcellsPV E6 protein degrades the tumor suppressor gene *p53* via the ubiquitin pathway which prevents apoptosis and enables cell proliferation and differentiation. HPV E7 protein activates the E2F transcription factor via the degradation of the retinoblastoma family of tumor suppressor proteins (*RB1, RBL1* and *RBL2*) (Bellacchio & Paggi, 2013). E2F transcription factors enhance the expression of various genes that are involved the cell proliferation, mainly the genes involved in the regulation of G1 and the S-phase of the cell cycle.

1.5 Cervical cancer screening

Tests for cervical cancer screening include HPV and Pap tests. A technical understanding of HPV assay is vital for the successful implementation of HPV-based screening because it is prudent to opt for a clinically validated test. The HPV DNA test is a multiplex assay that detects the DNA of high-risk HPV types, either directly by genomic detection or by polymerase chain reaction (PCR) amplification of a viral DNA fragment (Gheit et al., 2006). HPV genotyping identifies specific viral types (typically HPV 16 and 18), enabling the identification of those most at risk of persistence and progression. Onco-proteins E6 and E7 are detected in HPV mRNA tests, representing the integration of the virus. It's been 60 years since the Papanicolaou test (Pap) or cervical cytology became the mainstay of screening for cervical cancer, it is a simple and low-cost test (Comparetto & Borruto, 2015). Normal or negative Pap smear results indicate that no abnormal cells were found in the sample. Positive results on a Pap smear indicate abnormal cells were detected in the sample and may require additional treatment (Figure 2). A biopsy must be taken and confirmed by histology if the results are abnormal. Nearly half of all histological and cytologic diagnoses are the same. A cytology test has a sensitivity of 78% (range 30-87%) and a specificity of 62% (61-94%). Pap smears look for precancers, changes in the cervical wall that could lead to cervical cancer in the future if they are not treated. During the HPV test, the HPV virus that can cause these changes in cells is detected. DNA tests and RNA-based tests are available for the detection of high risk subtypes. The HC2 assay, approved by food and drug administration (FDA) in 2003, quantitatively detects the HPV subtypes. The Cervista 16/18 detects the HPV 16/18 types effectively. The Cobas 4800 System also detects the HPV-16 and HPV-18 subtypes along with HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-66, and HPV-68 (Goodman 2015). This test is based on PCR analysis, which can detect fewer than 10 copies of HPV DNA in a background of 1000 human cell DNA equivalents. The Aptima mRNA test is one of the RNA tests that detect E6 and E7 RNA and HPV 14 subtypes.

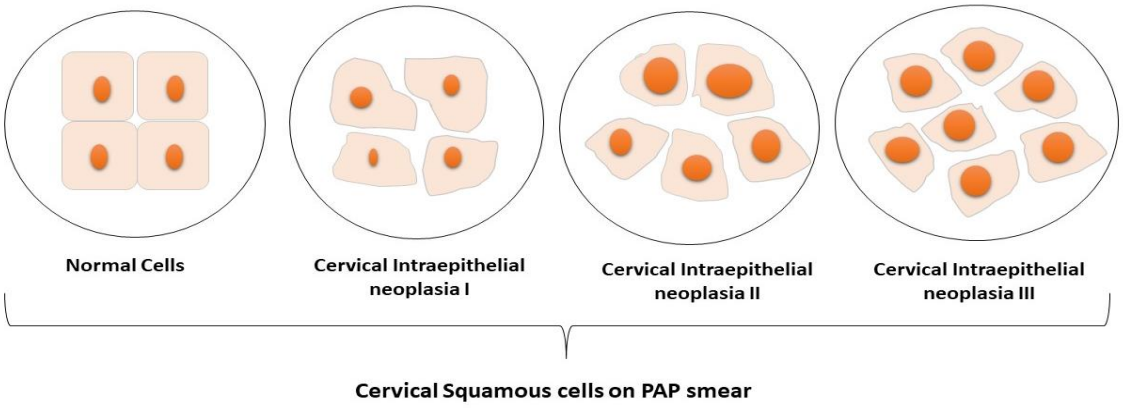

Figure 2. Cervical cancer Screening PAP smear: It is possible to categorize cervical intraepithelial neoplasia (CIN) through a Pap smear: CIN I (mild dysplasia), CIN II (moderate dysplasia), and CIN III (severe dysplasia and carcinoma in situ).

1.6 HPV vaccines

There are presently three FDA-licensed vaccines available to protect against HPV infection including HPV-16 and 18 such as the 9-valent HPV vaccine (Gardasil 9, 9vHPV), quadrivalent HPV vaccine (Gardasil, 4vHPV), and bivalent HPV vaccine (Cervarix, 2vHPV) respectively (Lin et al., 2020). Gardasil is the first approved HPV vaccine, which has been replaced by Gardasil 9. Another vaccine Cervarix is designed to prevent infection from HPV 16/18. These three vaccines are synthesized from virus-like particles of the L1 epitope (Deschuyteneer et al., 2010). The vaccine Gardasil 9 has more L1 epitope fraction against HPV 16/18 for inducing antibody-mediated immune responses than the vaccine Gardasil. Cervarix has the lowest L1 epitope fraction of the three vaccines but contains an efficient adjuvant, "Adjuvant System 04" (AS04) for improved immunogenicity. The AS04 mimics a Toll-like receptor 4 (TLR-4) to present antigens for inducing antibody-mediated immune responses.

2 Aim and Objectives

Aim

This study aims to analyze the severity of HPV-16/18 infection in cervical cancer and the impact of vaccines against HPV-16/18 strains through a systematic literature search. A previous meta-analysis related to this aim examined the severity of HPV 16 and the impact of HPV vaccines on the prevention of cervical cancer among Iranian women with normal cervical cytology, premalignant lesions, and invasive cervical cancer. The meta-analysis revealed that HPV vaccines prevent cervical cancer in Iran and they suggested that it will be "beneficial if current HPV vaccines are integrated into the national vaccination programs of Iran" (Jalilvand et al., 2015). This study demonstrated the importance of the HPV vaccine and the objectives raised from the above-mentioned research is reanalyzed in the present study to examine the severity of HPV-16/18 infection and the impact of HPV vaccines on the prevention of cervical cancer globally. The present study on the role of HVP-16/18 infection and the impact of various vaccines on HPV infection will provide great awareness regarding this to common people. The main objectives are,

1) To examine the severity of HPV-16/18 infection in cervical cancer
2) To evaluate the effectiveness of vaccines on HPV-16/18 variants against cervical cancer

3 Methodology

3.1 Systematic Review

In the present study, a systematic review regarding the severity-based analysis of HPV types was done priorly to examine the severity of HPV-16/18 infection in cervical cancer. Then a meta-analysis was conducted to evaluate the effectiveness of vaccines on HPV-16/18 variants against cervical cancer.

The systematic review process was performed by the eligibility criteria listed below:

- Studies in the English language discussing the severity of HPV types in cervical cancer patients (only human samples) were searched using the following databases from 1990 to 2021 through PubMed, Science Direct and Google Scholar.

- The specific articles were collected using the following search terms: "HPV 16/18/31/33/35/52/58 types in cervical cancer patients, HPV16/18 severity in CIN, HPV 16/18 in cervical lesions from the patients".
- Data extracted from the selected eligible articles to examine the severity of HPV-16/18 infection in cervical cancer.
- Finally summarizes the results of the individual studies to distinguish the severity of HPV-16/18 on cervical cancer over other HPV types.

The search strategy was depicted in Figure 3 Prisma flow chart for systematic review. The selected studies to perform systematic review are summarized in Table 1.

Table 1: Characteristics of the selected studies- Systematic review

Type of cervical cancer	Sample Size =n	HPV type	HPV 16/18 levels %	Study
Invasive cervical cancer	30848	HPV 16, 18 , 58, 33, 45, 31, 52, 35, 59, 39, 51 and 56.	70-76%	Li et al., 2011
Cervical intraepithelial neoplasia II and III	177	HPV 16, 18, 31, 33, 35, 45, 52, and 58	71%	Guo et al., 2007
Cervical intraepithelial neoplasia II and III	60	HPV-16,18 and 52	HPV 16= 36.2%, HPV 18= 15.5%	Ho et al., 2005
Cervical intraepithelial neoplasia II and III	99	HPV-16 and 18	HPV 16= 62% HPV 18=25%	Riethdorf et al., 2000
Cervical intraepithelial neoplasia III+	1467	HPV-16, 18, 31, 33, 35, 52 and 58	46.6%	Hosaka et al., 2013
Squamous intraepithelial lesions	718	HPV- 16, 6, 11, 18, and 33	35%,	Abba et al., 2003
Squamous intraepithelial lesions	2627	HPVs 6, 11, 16, 18, 31, 33, 35, 42, 43, 44, 45, 51, 52, 56, and 58	HPV 16= 47.1% HPV 18=26.8%	Lorincz et al., 1992
Cervical intraepithelial neoplasia II, III and adenocarcinoma *in situ*	8469	HPVs 6, 11, 16, 18, 31, 33, 45, 52, and 58	50%	Hariri et al., 2015
Invasive cervical cancer and Squamous intraepithelial lesions (SIL)	14595 ICC and 7094 SIL	HPV 16, 18, 31, 33, 35, 45, 52, and 58	70%	Smith et al., 2007

3.2 Meta-Analysis

Meta-analysis is the practice of systematically merging or synthesizing data from independent studies, focused on the same question, using statistical methods to calculate a total or absolute effect (Gurevitch et al. 2018). A meta-analysis can be conducted using the fixed-effect model or the random-effects model (Borenstein et al., 2010). Under the fixed-effect model, all studies in the analysis have exactly one true effect size, and all observed differences are the result of sampling error. Random-effect models are designed to estimate the mean of a distribution of true effects, not one true effect size across all studies. In a meta-analysis, study heterogeneity refers to the degree of variation in effect sizes between studies. The best meta-analyses not only provide an overall effect but also state the level of reliability of the estimate. To do this, the study heterogeneity needs to be quantified and analyzed. Due to the heterogeneity of data, random effects models have been largely used to ensure that data from different studies are included in the pooled estimate (Langan et al., 2019). Through meta-analysis, researchers combine different studies that focus on the same objectives to analyse the pooled outcome of the studies.

3.2.1 Search Strategy for Meta-analysis

The current study follows the random effect model to conduct the meta-analysis. In this study, clinical trials of invasive cervical cancer (only human samples) treated using HPV vaccines (Gardasil, Gardasil 9 and Cervarix) were searched manually from the following databases: Google Scholar, PubMed, and clinicaltrials.gov. The collected research articles mainly include invasive cervical cancer treated using HPV vaccines (Gardasil, Gardasil 9 and Cervarix), and also had to provide clear data on the use of polymerase chain reaction (PCR) technology to confirm HPV viral DNA; because the current study purely based on HPV 16/18 types. Furthermore, the collected clinical trials should strictly follow the ethical clearance protocols, otherwise, they will be excluded from the study. Based on the above criteria following points were extracted, sample size, year of publication, age, type of vaccine, the vaccine-specific prevalence of HPV16/18, HPV variants, and type of cervical specimen. Studies in the English language that were published from 2000 to 2021 were collected reporting the prevalence and prevention of HPV-16/18 infection in invasive cervical cancer. The process of evidence acquisition combined the following search terms: “HPV 16/18 vaccination in cervical cancer neoplasia”, “HPV 16/18 vaccination against cervical cancer randomized clinical trial” and “HPV vaccination against cervical cancer intraepithelial neoplasia randomized clinical trial”. Additional screening was performed by the reference lists from the relevant literature. Article abstracts and, where appropriate, full text of articles and cross-referenced studies identified from retrieved articles were screened for pertinent information. All duplicate records were removed. The search strategy was mentioned in the Figure 4 PRISMA flow chart for meta-analysis. The clinical trials for the meta-analysis were selected by the criteria listed below.

3.2.2 Eligibility Criteria

The publications were evaluated dependent on predefined inclusion and exclusion criteria. The inclusion criteria were as follows: randomised clinical trials published in English on HPV 16/18 vaccine efficacy in preventing cervical cancer (only human samples were included); prophylactic HPV vaccination versus no vaccination with approved ethical statements.

The exclusion criteria were as follows: case reports, case studies, editorials, review articles, and conference abstracts; studies testing newly developed HPV vaccines that have not received FDA approval, studies with non-human samples and clinical trials which failed to include approved ethical statements. The following items were extracted from each study: journal and year of publication, study design, study endpoint, study population (age and the number of patients), type of treatment, type of HPV vaccine (bivalent, quadrivalent, nonavalent), time of vaccination (before or after surgery), and duration of follow-up.

3.2.3 Data Extraction

To conduct the meta-analysis, the relevant articles were independently examined on the basis of their title, abstracts, and methods. The following keywords were used for searching articles: "treatment of HPV patients with cervical cancer", "HPV 16/18 infection in cervical cancer", "HPV vaccination in cervical cancer patients", "HPV vaccine effect against HPV 16/18", "HPV vaccination in cervical neoplasia patients", "prevalence of HPV 16/18 in cervical cancer", and "risk factors". After the search and inclusion and exclusion procedures, nine suitable articles were selected (figure 4). The data were extracted from nine articles (Figure 4). After the selection of articles, the data extracted for meta-analysis includes; HPV type, year of the study, participants' gender and age, study design, type of cervical cancer, HPV vaccine type, length of the follow-up period, the sample size for each group, and vaccine efficacy.

3.2.4 Data analysis

In this study, R Studio software (version 1.4.1717) was used for performing the meta-analysis. Meta-analysis was performed using the random effect models. Data were extracted in tabular form to generate the pooled odds ratios (table 2). The effectiveness of HPV 16/18 vaccines against cervical cancer was evaluated based on odds ratios and 95% confidence intervals. Pooled odds ratio and 95 percent confidence intervals for binary values were calculated.

The HPV vaccine efficacy on cervical cancer was evaluated by calculating odds ratios (OR) and using random effect models.

Vaccine Efficacy = (1-Risk in the vaccinated arm/Risk in the unvaccinated arm) x 100
Vaccine efficacy = (1-OR) x 100

The I^2 statistic was used to evaluate the statistically significant heterogeneity, which means $I^2=0$ implies there is no observed heterogeneity, and $I^2 \geq 50$ implies high heterogeneity as mentioned by the Cochrane Handbook. Forest plots are graphical representations used to illustrate results from individual studies and pooled analyses. The forest plot represents the association between studies included in a meta-analysis in relation to one another, showing the pooled effects of the studies and demonstrating heterogeneity among them. A funnel plot is used to investigate publication bias in meta-analyses. These scatterplots display the treatment effects from individual studies (horizontal axis) versus study size (vertical axis). A p-value of <0.05 was considered statistically significant.

4 Results

4.1 Systematic Review:

4.1.1 Selection of studies for Systematic review

The current study on the severity of HPV-16/18 infection in cervical cancer was analyzed via systematic review. A total of 172 articles were retrieved from Pubmed, science direct, and Google Scholar for the initial search. A Prisma flow chart depicting the process of article identification and selection process with relevant data was given in Figure 3. Twenty-four duplicate papers were removed. Ninty three articles that were not relevant were removed following the abstract screening. After the further screening of the full-text articles, 46 papers were removed due to studies involving non-human samples, using other treatment options, and review papers. Finally, nine studies that met the inclusion criteria were selected for the systematic review process, and the characteristics of the studies were included in Table 1.

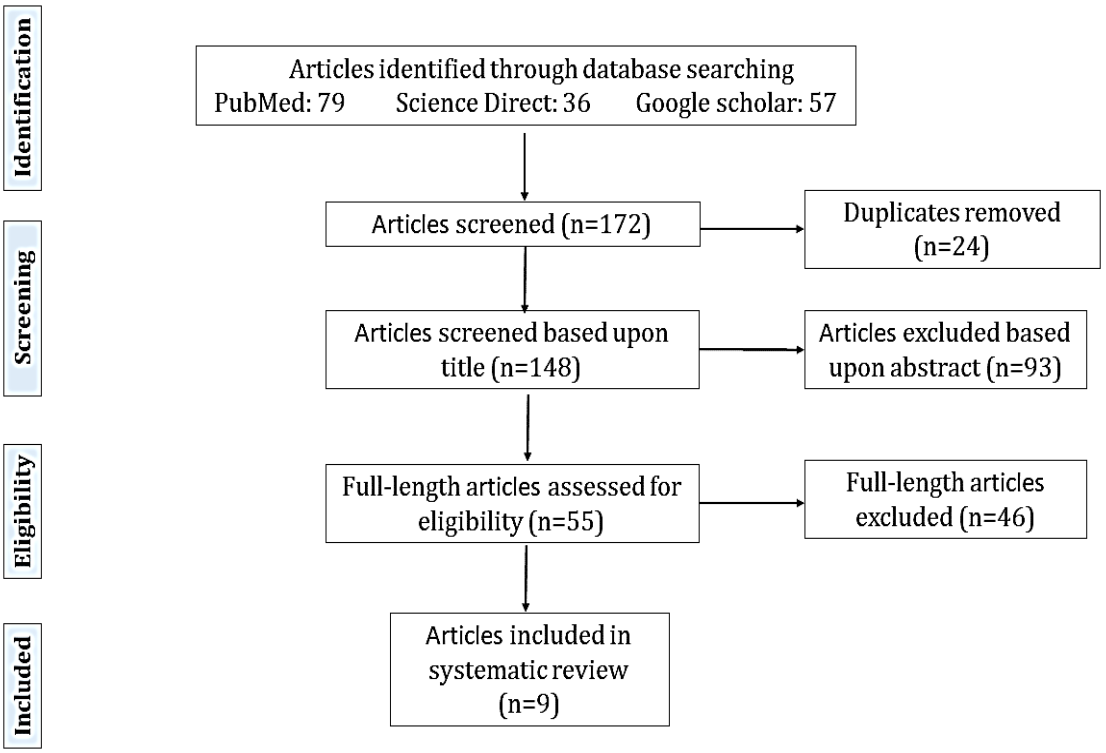

Figure 3. **PRISMA flow chart for systematic review:** PRISMA flow chart depicting the process of article identification, screening, eligibility and selected articles number with relevant data for systematic review.

4.1.2 Characteristics of the individual study- Systematic Review

The 9 studies included a total of 66154 cervical cancer cases, and the sample size for single studies ranges from 60 to 30848. The selected papers were published between 1990 to 2021. The list of studies were included in Table 1: Characteristics of the selected studies- Systematic review.

4.1.3 The severity of HPV-16/18 infection in cervical cancer

This systematic review describes the severity level of the HPV 16/18 virus causing cervical cancer. The overall database revealed that the HPV 16 variant causes severe metastasis and proliferation of cervical cancer. The study population consists of invasive cervical cancer, cervical intraepithelial neoplasia II II+, III, and III+, squamous intraepithelial lesions and adenocarcinoma *in situ.* The highest rate of HPV 16/18 types was detected in severe CIN II, II+, III, and III+ lesions. The positivity rate of HPV 16/18 steadily increased from CIN II, II+, III, and III+ lesions. The outcome of the high sample size included studies shows that more than 50% of cervical cancer lesions are HPV 16/18 positive (Hariri et al.,(2015); Lorincz et al., (1992); Hosaka et al., (2013); Riethdorf et al., (2000) and Ho et al., (2005)). Among the 9 studies (Smith et al., (2007); Guo et al., (2007) and Li et al., (2011) showed that more than 70% of invasive cervical cancer were attributed to HPV 16/18 variants. The overall results emphasise the hypothesis that the HPV 16/18 variants were the dominant HPV types causing severe cervical cancer globally.

4.2 Meta-analysis

4.2.1 Selection of studies for meta-analysis

The present study of meta-analysis focuses on evaluating the effectiveness of vaccines on HPV-16/18 variants against cervical cancer. A total of 95 papers were identified, out of which Seven articles were overlapping between PubMed and Google scholar. Sixty-four articles were excluded as they were not clinical trials but reviews, comments on HPV vaccines, and letters to the editor. The remaining twenty-four articles were chosen for a full-text evaluation. Finally, nine articles of randomized control trials were included to develop the HPV 16/18 vaccine efficacy meta-analysis depicted in Figure 4. The selected studies included were randomized multicentered phase III and II controlled clinical trials. A total of 78308 women of ages 15-26 years old (39124 CIN cases and 39184 control cases) from the selected trials were comprised in this meta-analysis of HPV 16/18 vaccine efficacy (Table 2).

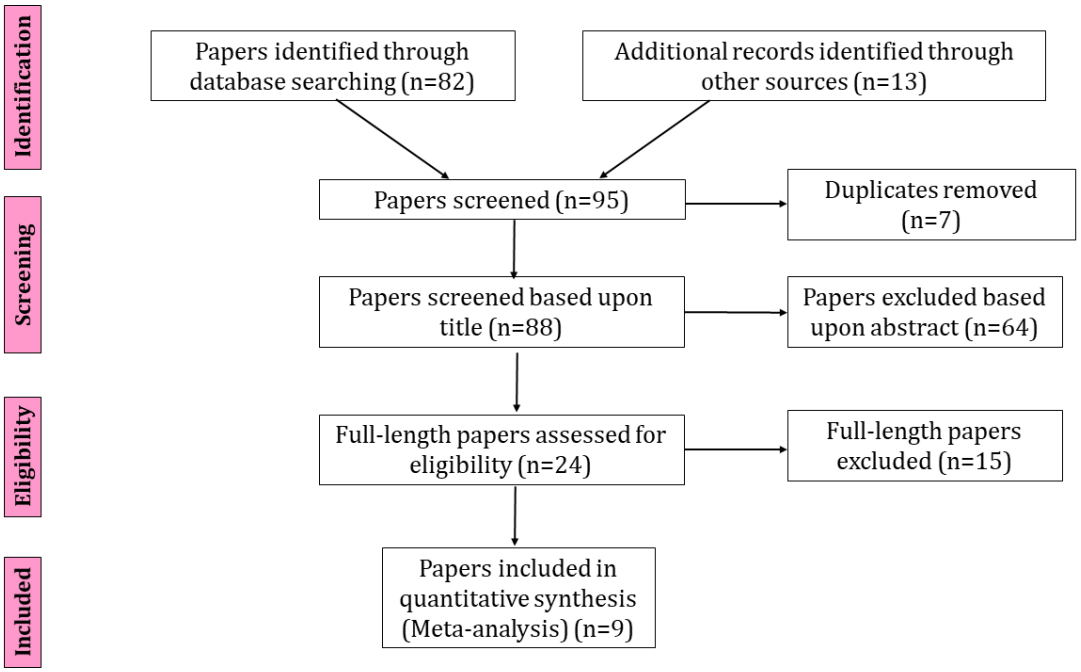

Figure 4. **PRISMA flow chart for meta-analysis:** PRISMA flow chart depicting the process of clinical trial article identification, screening, eligibility and selected articles number with relevant data for meta-analysis.

4.2.2 Characteristics of the individual study-Meta- analysis

Altogether nine randomized multicentered, double-blind phases III and II controlled clinical trials were included in this meta-analysis. The list of studies was included in table 2- Characteristics of the selected studies- Meta-analysis.

Table 2: Characteristics of the selected studies- Meta-analysis

Study	Study Design	Age group	Type of Cervical cancer	HPV Type	Type of HPV Vaccine	Duration of the HPV Vaccine Dose	Sample Size Vaccine = n	Sample Size control = n	Odds Ratio	Vaccine efficacy	Confidence interval (CI)
Paavonen et al., 2007	Phase III double-blind, randomized controlled trial	15–25 years	Cervical intraepithelial neoplasia (CIN) 2+	HPV16 or HPV18	Adjuvanted HPV16/18 vaccine	Three-dose vaccination schedule (0, 1, and 6 months)	7788	7838	0.10	90.40%	97·9% CI (53·4–99·3), p<0·0001).
Harper et al., 2004	Randomized, double-blind, controlled trial	15–25 years	Cervical intraepithelial neoplasia (CIN) 1 and 2	HPV16 or HPV18	Bivalent HPV-16/18 virus-like particle vaccine	Three-dose vaccination schedule (0, 1, and 6 months)	560	553	0.07	92.90%	95% CI (70·0–98·3); p<0·0001)
Lehtinen et al., 2012	Randomized, double-blind PATRICIA trial	15–25 years	Cervical intraepithelial neoplasia (CIN) 3+	HPV-16/18	HPV-16/18 AS04-adjuvanted vaccine (Cervarix, GlaxoSmithKline Biologicals)	Three-dose vaccination schedule (0, 1, and 6 months)	8694	8708	0.37	62.9%	95% CI (54·1 - 70·1) P<0.001)
Ault, 2007	A Randomized Controlled Trial	16–26 years	Cervical intraepithelial neoplasia (CIN) 2/3	HPV-16/18	Quadrivalent vaccine GARDASIL	Three-dose vaccination schedule, Day 1, month 2, and month 6	8579	8550	0.01	99%	95% CI (93–100), P<0.001)
Herrero et al., 2011	Double-blind randomized controlled trial	18 - 25 years	Cervical neoplasia	HPV-16/18	Bivalent ASO4-adjuvanted HPV16/18 vaccine (Cervarix)	Three-dose vaccination schedule (0, 1, and 6 months)	2635	2677	0.09	90.90%	95% CI, (82.0–95.9), P<0.001)
Mao et al., 2006	A Randomized Controlled Trial	16 –23 years	Cervical intraepithelial neoplasia (CIN) 2–3	HPV-16	HPV16 L1 virus-like particle vaccine	Three-dose vaccination schedule, Day 1, month 2, and month 6	755	750	0.02	100%	95% CI (84 – 100%), P<0.001)
Joura et al., 2007	Randomized Controlled Trial	16–26 years	CIN II/III	HPV6/11/16/18	Quadrivalent HPV6/11/16/18	Three-dose vaccination schedule, Day 1,	9087	9087	0.29	71%	95% CI (37–88), P<0.001)

					L1 virus-like-particle vaccine	month 2, and month 6					
Villa et al., 2005	Randomised double-blind placebo-controlled multicentre phase II efficacy trail	16–23 years	Cervical intraepithelial neoplasia (CIN)	HPV6/1 1/16/18	Quadrivalent HPV (type 6, 11, 16, and 18) L1 VLP vaccine	Three-dose vaccination schedule, Day 1, month 2, and month 6	258	256	0.06	100%	95% CI (32–100), p=0·0072
Koutsky et al., 2002	A Randomized Controlled Trial	16–23 years	Cervical intraepithelial neoplasia (CIN)	HPV-16	HPV-16 L1 virus-like–particle vaccine	Three-dose vaccination schedule (0, 2, and 6 months)	768	765	0.01	100%	95% CI, (90 -100), P<0.001

4.2.3 Efficacy of HPV 16/18 vaccines in Cervical cancer

In the period between 2000-2021, nine relevant studies were selected on CIN I to CIN III patients. The present meta-analysis evaluates by random effects model in 95% CI was calculated using a forest plot. The HPV 16/18 vaccines showed prophylactic efficacy against cervical cancer (CIN I-III) and thus be used for cervical cancer prevention associated with HPV 16 or HPV 18. The HPV vaccine was bivalent against HPV 16 and 18 genotypes in five studies, and quadrivalent in four studies. All studies assess the recurrence of HPV infection and CIN within 6, 12, and 18 months after vaccine treatment. In the nine studies included, CIN (I-III), 39124 participants underwent HPV 16/18 vaccine treatment, while other 39184 participants underwent placebo or control hepatitis A vaccine. In all, 143 (0.36%) participants in the HPV 16/18 vaccine-treated group and 649 (1.65%) in the control group showed CIN recurrence. A strong vaccine-induced antibody response for both HPV 16/18 was detected in all studies. Based on the results, the vaccine was effective, well tolerated, and immunogenic in a broad range of young and adult women. This supports its potential use to prevent CIN and cervical cancer.
Heterogeneity for this meta-analysis was found to be I^2= 80% (*p-value* <0.01). The meta-analysis showed that differences in the vaccination sources, publication year and participant number may be the factor responsible for heterogeneity. The overall Odds Ratio (OR) was observed at 0.09 (95% CI= 0.04-0.20) for the random effect model (figure 5). The cumulative vaccine efficacy calculated was found to be 91%.

Cumulative Vaccine efficacy = (1-0.09)x100= 91%

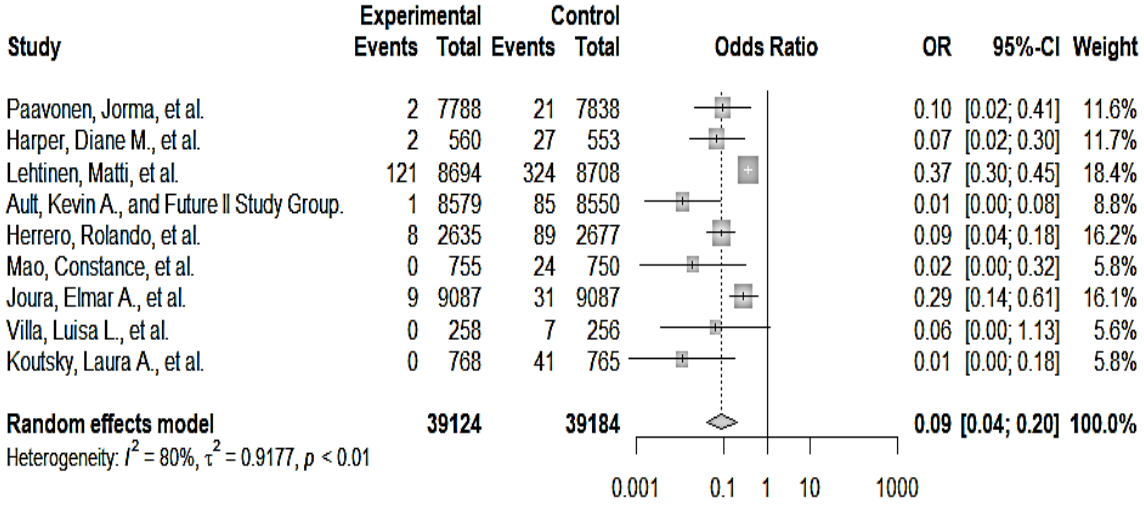

Figure 5. Forest plot of the meta-analysis of HPV vaccine efficacy in preventing cervical cancer: The overall OR was observed at 0.09 (95% CI= 0.04-0.20) for the random effect model. Heterogeneity was found to be I^2= 80% (*p-value* <0.01).

4.2.4 Risk of bias assessment

Totally 9 studies were selected to assess the HPV 16/18 vaccine efficacy in cervical cancer patients. To assess the publication bias in this meta-analysis, funnel plots were generated. In this plot, the Odds ratios are plotted against their standard error. As long as there is no publication bias, the observed studies should be distributed symmetrically around the pooled effect size. Figure 6 showed the asymmetrical distribution of data which indicates some evidence of publication bias.

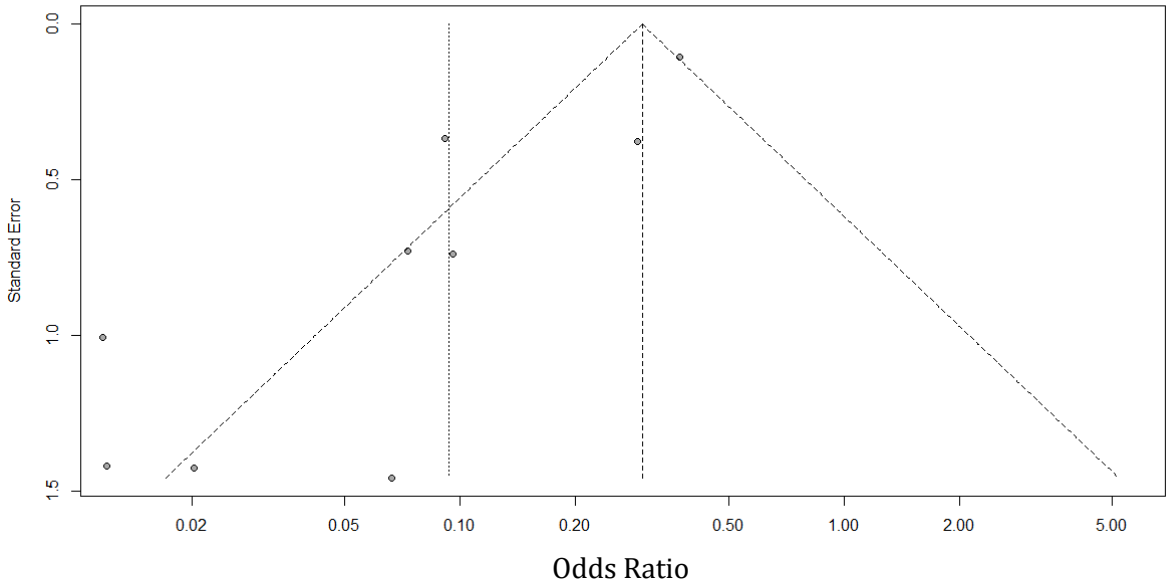

Figure 6. **Funnel plot**: To assess the publication bias in this meta-analysis. Asymmetrical distribution of data indicates some evidence of publication bias.

5 Discussion

HPV is an epithelium tropic DNA virus that belongs to the family of papovaviridaeviruses. According to epidemiology and laboratory studies, HPV 16 is the most potent carcinogen among all high-risk types, and HPV 16 and 18 combined account for 70% of cervical cancers, as well as many other types of HPV-related cancers (Kovacevic et al., 2019). Invasive cervical cancers are usually preceded by asymptomatic lesions that are caused by the same high-risk HPV types that are associated with cervical cancer. Globally, the high-risk type of HPV 16/18 causes 70% of cervical cancer (CIN I -III), due to the presence of E5, E6, and E7 oncoprotein (Ramakrishnan et al., 2015). The E6 and E7 proteins inhibit the tumor suppressor genes like *p53* and *pRb*. Simultaneously E5 oncoprotein enhances the activity of epidermal growth factor receptors for the suppression of major histocompatibility complex activation (Balasubramaniam et al., 2019).

The current study was performed in a way to provide a better insight into the severity of the HPV 16/18 genotype to cause cervical cancer and the importance of preventing such a serious issue by taking the HPV vaccination. The present study demonstrates a comprehensive systemic review and meta-analysis of the infection and severity of HPV 16/18 and the protective effect of HPV vaccination on cervical cancer. Systematic review analysis covers the most suitable 9 articles to examine the severity of HPV-16/18 infection in cervical cancer. Besides HPV16 and HPV18, the genotypes HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 are also strongly associated with cervical cancer. The findings of the systematic review point out that the HPV 16/18 variant has high oncogenic properties than other types. majority of CIN II, II+, III, and III+ lesions contain HPV 16/18 infection. They found that more than 70% of the invasive cervical cancer were attributed to either HPV 16 or 18 (Smith et al. 2007; Guo et al., 2007; Li et al., 2011). The results were supported by the findings of Guan et al. (2012) which examined that the incidence of HPV-16 induced cervical cancer levels increased steeply from normal/ASCUS/LSIL/CIN1/CIN2/HSIL (40/47%) to CIN3/ICC (58/63%). It is likely that HPV-16 causes cervical cancer at a faster rate than other HPV types from low-grade squamous intraepithelial lesions to high-grade squamous

intraepithelial lesions, as shown in the study by Jalilvand et al. (2015) by the severity ratio of HPV 16 in ICC. According to the findings of Castle et al. (2005), HPV16 was the most prevalent type of HPV among women with atypical squamous cells of undetermined significance (ASCUS) (14.9%, 95% CI = 13.7% to 16.1%) and women with the low-grade squamous intraepithelial lesion (LSILs) (21.1%, 95% CI =19.1% to 23.2%). The specific knowledge of HPV types would help the vast development of HPV-DNA-based screening tests across the world and the application of prophylactic vaccinations worldwide.

The Pap test is widely used for the screening of cervical cancer cytology, the test began over 50 years ago. However, "prevention is better than cure", taking a prophylactic vaccine against the high-risk and low-risk HPV types will protect against HPV-associated cervical cancer. HPV vaccinations are designed to prevent invasive cervical cancer by preventing infection with major oncogenic HPV types. According to the randomized control trials, HPV vaccine efficacy was found to be 90-100% against cervical cancer. HPV vaccination was found to reduce post-surgical recurrent disease both in women and men with previous HPV infections based on several studies (Michalczyk et al., 2022). The FDA approved the tetravalent vaccine for use in girls and women from 9 to 26 years of age on 8 June 2006, and the Advisory Committee on Immunization Practices recommended routine vaccinations for girls aged 11 to 12 (Liu, 2022). Vaccines directed against HPV 16/18 were sanctioned by European Commission for the commercial production of the tetravalent vaccine on 20th September 2006 (Barr & Sings, 2008). Malagon et al. (2012) compared the cross-protective role of the bivalent HPV 16/18 vaccine and the quadrivalent HPV (6,11, 16 and 18) vaccine against persistent HPV infection and CIN through meta-analysis. They found that the bivalent vaccine shows high efficacy than the quadrivalent vaccine.

The meta-analysis part of the study selected the clinical trials with a sample size that included cervical cancer patients, particularly HPV 16/18 infection to evaluate the effectiveness of HPV vaccines. Clinical trial studies commonly use odds ratios as a measure of a study's success. Using odds ratios, it is possible to predict the likelihood that a certain event will occur as a result of exposure (Tenny et al., 2017). Hence, the higher odds ratio (greater than 1) indicates that a larger number of events (here, the occurrence of cervical cancer after the vaccination follow-up period) occurred in the treatment group. If the odds ratio is less than 1, the event is less likely to occur with the treatment group. The meta-analysis shows the cumulative efficacy of HPV vaccination in different studies was 91% in 95 % CI and the odds ratio was found to be 0.09 (95% CI= 0.04;0.20). The odds ratio is less than 1, indicating less occurrence of cervical cancer in patients after vaccination. In other words, overall, data suggest that the HPV-16/18 vaccine is highly effective at preventing HPV-16/18-associated cervical infection, cytological abnormalities, and histological abnormalities. Heterogeneity for this meta-analysis was found to be I^2= 80% by a *p*-value <0.01. Heterogeneity in meta-analyses is generally defined as the difference between results from different studies. The heterogeneity is due to the use of different sources of vaccination histories from various studies. Based on Begg's method and Egger's method, the funnel plot asymmetry in meta-analyses was generally greater with fewer literature databases searched. Hence, the result of the funnel plot showed some evidence of publication bias, due to fewer articles. In this meta-analysis, the funnel plot shows evidence of publication bias due to asymmetrical distribution.

The present meta-analysis included nine studies with 77519 more participants than the previous study of meta-analysis by Ventue et al., (2022) and confirmed that HPV vaccination reduces and prevents HPV 16/18-associated cervical cancer. In this study, Paavonen et al. (2007) found that the vaccine efficacy against HPV16/18 induced CIN2+ was 90.4% (97.9% CI 53.4–99.3; p<0.0001). Harper et al. (2004) demonstrated that the vaccine efficacy was 92.9% (70.0–98.3) against cervical cancer associated with HPV-16/18 infection. Ault (2007) reported that the vaccine efficacy was found to be 99% (95% CI 93–100, P<0.001). In particular, three studies (Mao et al., 2006; Villa et al., 2005; Koutsky et al., 2002) reported 100% vaccine efficacy, in which

vaccination provides high protection against HPV 16/18 infection and reduces the levels of associated cervical cancer. Another four studies (Harper et al., 2004; Ault, 2007; Herrero et al., 2011) reported above 90% vaccine efficacy against HPV infection and reduce the incidence of HPV 16/18 associated cervical cancer.

The current study evaluated the therapeutic efficacy of HPV vaccines mainly in the prevention of HPV 16/18 infection. The lowest vaccine efficacy among the nine studies (63%) for HPV-16/18 AS04-adjuvanted vaccine was observed in Lehtinen et al. (2012) in CIN 3+ patients and the highest vaccine efficacy (100%) was found in three studies by Mao et al. (2006), Villa et al. (2005) and Koutsky et al. (2002) in CIN I to CIN II patients, which stated in table 2. From the forest plot (figure 5) of meta-analysis, it is clear that all nine selected studies favour the vaccine treatment method (left arm). The studies include vaccine types of prophylactic adjuvanted bivalent L1 virus-like-particle vaccine, HPV-16/18 AS04-adjuvanted vaccine (Cervarix, and GlaxoSmith), and Quadrivalent vaccine GARDASIL. Compared to older women, younger women are better able to respond to the HPV vaccine because they receive it at a younger age (10-14 years) and thus receive earlier protection against infection. The selected randomized clinical trials have shown that women in the age group 18–30 years also have a strong immune response to the HPV vaccine, producing high levels of virus-neutralizing antibodies.

This study provides evidence for the severity of persistent HPV 16/18 infection for the development of cervical cancer. It was noted that the incidence of HPV 16/18 infection was common and high in young women (15-25 years) as reported in previous studies (Ramanakumar et al., 2016). The results of our study indicate that immunization of women in early adolescence will provide the largest population benefit since HPV infections begin at sexual debut and are high in adolescents, which means that vaccinations of girls before sexual debut are most likely to achieve the greatest population benefit.

HPV vaccines are often viewed as cancer prevention vaccines, but they may pose unique challenges since parents may have difficulty reconciling the fact that 9–13-year-old girls will not develop cancer for at least two to three decades if they are vaccinated (Maine et al., 2011). Naud et al. (2014) reported that the bivalent vaccines provide long-term protection against HPV 16/18 infection for around 9.4 years post-vaccination. Increasing evidence indicates that HPV vaccines reduce HPV severity and associated diseases, such as warts, dysplastic cervical tissue, and cervical cancer. Bruni et al. (2016) estimated that about 118 million women had been vaccinated against HPV globally, which reduces the rate of cervical cancer.

Two large-scale, double-blind randomized controlled trials of bivalent vaccination, the Costa Rica Vaccine Trial (NCT00128661) (Hildesheim et al., 2014) and PATRICIA trial (NCT001226810) (Apter et al., 2015) demonstrated that 4 years after vaccination against cervical infection caused by HPV 16 and HPV 18, one and two doses of bivalent vaccine provided similar protection to the complete three-dose schedule against cervical infections. In order to prevent the spread of infection, the WHO recommends two doses in children between 9-12 years of age, those who have reached 15 by the time of the second dose are also covered by two doses (World Health Organization, 2017). Powell et al. (2012) investigated the impact of HPV vaccination in women with CIN2+. A total of 3850 women had vaccination history of which 1900 women had documented vaccination history among the 5083 CIN cases. A significantly lower proportion of CIN2+ lesions due to HPV 16/18 was observed in women whose vaccination started >24 months before their trigger Pap than in those who did not receive vaccination, the prevalence ratio (PR) equals, aPR = .67 (95% CI: 0.48–0.94) compared to vaccinated on 1-12 months before trigger Pap, aPR = 0.93 (95% CI: 0.77–1.13).

The present study has some limitations. The findings may have been biased by the use of different sources to gain vaccination history, as sources and project sites varied in terms of completeness and accuracy. Since 30% of cervical cancers are caused by other factors which were not covered by the HPV vaccines and the vaccine effect would be effective in three decades, hence, vaccination

does not eliminate the need to screen women. Overall, these results suggest that 70% of cervical cancer was caused by either HPV 16 or HPV 18 and a prophylactic vaccine against HPV16/18 prevents HPV-associated cervical cancer.

6. Future Research

In this study, HPV vaccine efficacy for the prevention of cervical cancer was examined through nine randomized clinical trials. More research is needed to implement the importance of HPV vaccination globally. Bhatla et al (2008) also suggest the same criteria that the integration of FDA-accepted HPV vaccines into national vaccination programs is beneficial. Further, the recurrence of HPV infection and cervical cancer after the vaccination program needs to be assessed as a long-term screening process. It would be possible to significantly reduce the global burden of cervical cancer through vaccination in young women and low-cost HPV DNA screening in older women, but such an approach would require a demonstration project before widespread adoption could occur (Lowy et al., 2012). The benefits of the vaccine must be optimized for each individual and for the entire population over time. The selected nine studies calculated the vaccine follow-up in a short time period of up to 2-4 years (Harper, Diane M., et al. and Herrero, Rolando, et al. 2011). In order to determine whether booster doses will be necessary, longer follow-up studies will be needed to assess the duration of efficacy of the HPV 16/ 18 vaccine. And a combined large-scale study is needed for screening the HPV vaccine efficacy all over the world to implement the importance of HPV vaccination among people. In the future, cancer immunoprevention through vaccination will be a standard part of long-term cancer treatment.

7. Ethical aspects

The term "meta-analysis" refers to a method of synthesizing research. The method involves statistically integrating data from similar but separate studies, usually using summary statistics from research reports. Meta-analysts (a) collect as many published and unpublished reports addressing a topic as possible, (b) extract effect sizes from the reports, (c) combine the effect sizes to calculate the average effect size and the associated confidence interval, and (d) examine study features that might influence the study outcome based on the sample and study characteristics (Cooper & Dent, 2011). It is essential that meta-analysis and systematic reviewers, like primary researchers, engage reflexively with numerous ethical issues associated with potential conflicts of interest. A systematic review and meta-analysis are frequently cited in documents that influence educational policy and practice. In the meta-analysis, a reviewer must ensure that the perspectives of authors and participants in original studies are represented. This is done so that the missing perspectives can be perceived. It is critical to scrutinize the domain of applicability of meta-analysis in order to prevent unintended extrapolations to domains where they are not applicable. The interpretive meta-analysis should strive to present an authentic reflection of the perspectives of the original participants as expressed through the authors' interpretive lenses. Rather than aiming for generalizability, they should focus on how individual findings intersect with their methodological and contextual configurations to achieve transferability. Through critical engagement with the relevant research, participatory systematic reviews can improve the local world experience of participants (Suri et al., 2020).

During the study, we do not have direct access to participants of primary research studies included in the meta-analysis. As a reviewer, it is important to ethically consider the quality and relevance of evidence reported in primary studies. It is important to use evaluation criteria that are commensurate with the epistemological positioning of the study's author in evaluating the quality of evidence in individual reports (Major et al., 2012). It's more ethical to evaluate the ethical impact of individual studies, rather than selecting perfect studies. Meta-analysis is frequently read and cited in various studies which influence educational policy and practice.

Hence, ethical considerations associated with clinical trials have serious implications. The present meta-analysis addresses very serious scientific questions to those for which the data were collected (and to which patients already gave consent). Hence, the previous studies collected strictly followed proper ethical protocols.

Incorporating ethical considerations into meta-analyses will increase research workers' awareness of the need for ethical research. Results may also be more accurate if ethical considerations are incorporated (Weingarten et al., 2004). In this study, the published articles which fail to include ethical approval forms were excluded through the screening process. The present study addresses a very serious issue regarding the impact of HPV-16/18 infection on cervical cancer patients, so consent from each patient in the selected clinical trial is important. Such clinical trials only come up to the standard levels while interpreting the quality of the result. Because the present study on the role of HVP-16/18 infection and the impact of various vaccines on HPV infection will provide great awareness regarding this to common people.

8. Conclusion

In this study, we used data from a large population-based system for monitoring the effect of HPV vaccination on cervical cancer patients to examine HPV vaccine effectiveness on HPV 16/18 types in CIN2+ lesions. Nine relevant studies with a total of 78308 women participants were selected via searching various databases such as Google Scholar, PubMed, and clinicaltrials.gov. The HPV vaccine efficacy on cervical cancer was evaluated by calculating odds ratios (OR) and using random effect models. Statistical analyses were performed using mean differences or pooled odds ratios (OR) with 95% confidence intervals (95% CI). Publication bias was examined using the funnel plot graph. The HPV vaccination reduces the severity of cervical cancer (OR 0.09; 95% CI= 0.04-0.20; $p<0.01$). The results support that a prophylactic vaccine against HPV16/18 prevents the severity of HPV-associated cervical cancer. HPV16-positive women face a higher absolute risk for cervical precancer compared to HPV16-negative women. In conclusion, this meta-analysis points to the importance of introducing HPV vaccination programs all over the world.

9. References

Abba, M. C., Gomez, M. A., & Golijow, C. D. (2003). Human papillomavirus genotype distribution in cervical infections among woman in La Plata, Argentina. *Revista Argentina de Microbiologia*, *35*(2), 74-79.

Apter, D., Wheeler, C. M., Paavonen, J., Castellsagué, X., Garland, S. M., Skinner, S. R., ... & Dubin, G. (2015). Efficacy of human papillomavirus 16 and 18 (HPV-16/18) AS04-adjuvanted vaccine against cervical infection and precancer in young women: final event-driven analysis of the randomized, double-blind PATRICIA trial. *Clinical and Vaccine Immunology*, *22*(4), 361-373.

Ault, K. A. (2007). Effect of prophylactic human papillomavirus L1 virus-like-particle vaccine on risk of cervical intraepithelial neoplasia grade 2, grade 3, and adenocarcinoma in situ: a combined analysis of four randomised clinical trials. *The Lancet*, *369*(9576), 1861-1868.

Balasubramaniam, S. D., Balakrishnan, V., Oon, C. E., & Kaur, G. (2019). Key molecular events in cervical cancer development. Medicina, 55(7), 384.

Barr, E., & Sings, H. L. (2008). Prophylactic HPV vaccines: new interventions for cancer control. *Vaccine*, *26*(49), 6244-6257.

Basu, P., Mittal, S., Vale, D. B., & Kharaji, Y. C. (2018). Secondary prevention of cervical cancer. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *47*, 73-85.

Bellacchio, E., & Paggi, M. G. (2013). Understanding the targeting of the RB family proteins by viral oncoproteins to defeat their oncogenic machinery. *Journal of cellular physiology*, *228*(2), 285-291.

Belnap, D. M., Olson, N. H., Cladel, N. M., Newcomb, W. W., Brown, J. C., Kreider, J. W., ... & Baker, T. S. (1996). Conserved features in papillomavirus and polyomavirus capsids. *Journal of molecular biology*, *259*(2), 249-263.

Bhatla, N., Lal, N., Bao, Y. P., Ng, T., & Qiao, Y. L. (2008). A meta-analysis of human papillomavirus type-distribution in women from South Asia: implications for vaccination. *Vaccine*, *26*(23), 2811-2817.

Borenstein, M., Hedges, L. V., Higgins, J. P., & Rothstein, H. R. (2010). A basic introduction to fixed-effect and random-effects models for meta-analysis. *Research synthesis methods*, *1*(2), 97-111.

Boulet, G., Horvath, C., Broeck, D. V., Sahebali, S., & Bogers, J. (2007). Human papillomavirus: E6 and E7 oncogenes. *The international journal of biochemistry & cell biology*, *39*(11), 2006-2011.

Bradford, L. E. S. L. I. E., & Goodman, A. N. N. E. K. A. T. H. R. Y. N. (2013). Cervical cancer screening and prevention in low-resource settings. *Clinical Obstetrics and Gynecology*, *56*(1), 76-87.

Bruni, L., Diaz, M., Barrionuevo-Rosas, L., Herrero, R., Bray, F., Bosch, F. X., ... & Castellsagué, X. (2016). Global estimates of human papillomavirus vaccination coverage by region and income level: a pooled analysis. *The Lancet Global Health*, *4*(7), e453-e463.

Castle, P. E., Solomon, D., Schiffman, M., & Wheeler, C. M. (2005). Human papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. *Journal of the National Cancer Institute*, *97*(14), 1066-1071.

Ciapponi, A., Bardach, A., Glujovsky, D., Gibbons, L., & Picconi, M. A. (2011). Type-specific HPV prevalence in cervical cancer and high-grade lesions in Latin America and the Caribbean: systematic review and meta-analysis. *PloS one*, *6*(10), e25493.

Comparetto, C., & Borruto, F. (2015). Cervical cancer screening: A never-ending developing program. *World Journal of Clinical Cases: WJCC*, *3*(7), 614.

Cooper, H., & Dent, A. (2011). Ethical issues in the conduct and reporting of meta-analysis. *Handbook of ethics in quantitative methodology*, 417-443.

Denny, L., Quinn, M., & Sankaranarayanan, R. (2006). Screening for cervical cancer in developing countries. *Vaccine*, *24*, S71-S77.

Deschuyteneer, M., Elouahabi, A., Plainchamp, D., Plisnier, M., Soete, D., Corazza, Y., ... & Deschamps, M. (2010). Molecular and structural characterization of the L1 virus-like particles that are used as vaccine antigens in Cervarix™, the AS04-adjuvanted HPV-16 and-18 cervical cancer vaccine. *Human vaccines*, *6*(5), 407-419.

Di Donato, V., Caruso, G., Petrillo, M., Kontopantelis, E., Palaia, I., Perniola, G., ... & Bogani, G. (2021). Adjuvant HPV vaccination to prevent recurrent cervical dysplasia after surgical treatment: a meta-analysis. *Vaccines*, *9*(5), 410.

Didierlaurent, A. M., Morel, S., Lockman, L., Giannini, S. L., Bisteau, M., Carlsen, H., ... & Garçon, N. (2009). AS04, an aluminum salt-and TLR4 agonist-based adjuvant system, induces a transient localized innate immune response leading to enhanced adaptive immunity. *The Journal of immunology*, *183*(10), 6186-6197.

Faridi, R., Zahra, A., Khan, K., & Idrees, M. (2011). Oncogenic potential of Human Papillomavirus (HPV) and its relation with cervical cancer. *Virology Journal*, *8*, 1-8.

Gheit, T., Landi, S., Gemignani, F., Snijders, P. J., Vaccarella, S., Franceschi, S., ... & Tommasino, M. (2006). Development of a sensitive and specific assay combining multiplex PCR and DNA microarray primer extension to detect high-risk mucosal human papillomavirus types. *Journal of clinical microbiology*, *44*(6), 2025-2031.

Gibson, E. J., Bell, D. L., & Powerful, S. A. (2014). Common sexually transmitted infections in adolescents. *Primary care: clinics in office practice*, *41*(3), 631-650.

Goodman, A. (2015). HPV testing as a screen for cervical cancer. *Bmj*, *350*.

Guan, P., Howell-Jones, R., Li, N., Bruni, L., De Sanjosé, S., Franceschi, S., & Clifford, G. M. (2012). Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. *International journal of cancer*, *131*(10), 2349-2359.

Guo, M., Sneige, N., Silva, E. G., Jan, Y. J., Cogdell, D. E., Lin, E., ... & Zhang, W. (2007). Distribution and viral load of eight oncogenic types of human papillomavirus (HPV) and HPV 16 integration status in cervical intraepithelial neoplasia and carcinoma. *Modern Pathology*, *20*(2), 256-266.

Gurevitch, J., Koricheva, J., Nakagawa, S., & Stewart, G. (2018). Meta-analysis and the science of research synthesis. *Nature*, *555*(7695), 175-182.

Hariri, S., Unger, E. R., Schafer, S., Niccolai, L. M., Park, I. U., Bloch, K. C., ... & Markowitz, L. E. (2015). HPV type attribution in high-grade cervical lesions: assessing the potential benefits of vaccines in a population-based evaluation in the United States. *Cancer Epidemiology, Biomarkers & Prevention*, *24*(2), 393-399.

Harper, D. M., Franco, E. L., Wheeler, C., Ferris, D. G., Jenkins, D., Schuind, A., ... & Dubin, G. (2004). Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *The lancet*, *364*(9447), 1757-1765.

Herrero, R., Wacholder, S., Rodríguez, A. C., Solomon, D., González, P., Kreimer, A. R., ... & Hildesheim, A (2011). Prevention of persistent human papillomavirus infection by an HPV16/18 vaccine: A community-based randomized clinical trial in Guanacaste, Costa Rica. Cancer Discov. 2011; 1: 408–419. doi: 10.1158/2159-8290.

Hildesheim, A., Wacholder, S., Catteau, G., Struyf, F., Dubin, G., Herrero, R., & CVT Group. (2014). Efficacy of the HPV-16/18 vaccine: final according to protocol results from the blinded phase of the randomized Costa Rica HPV-16/18 vaccine trial. *Vaccine*, *32*(39), 5087-5097.

Ho, C. M., Yang, S. S., Chien, T. Y., Huang, S. H., Jeng, C. J., & Chang, S. F. (2005). Detection and quantitation of human papillomavirus type 16, 18 and 52 DNA in the peripheral blood of cervical cancer patients. *Gynecologic oncology*, *99*(3), 615-621.

Hosaka, M., Fujita, H., Hanley, S. J., Sasaki, T., Shirakawa, Y., Abiko, M., ... & Sakuragi, N. (2013). Incidence risk of cervical intraepithelial neoplasia 3 or more severe lesions is a function of human papillomavirus genotypes and severity of cytological and histological abnormalities in adult Japanese women. *International journal of cancer*, *132*(2), 327-334.

Jalilvand, S., Shoja, Z., Nourijelyani, K., Tohidi, H. R., & Hamkar, R. (2015). Meta-analysis of type-specific human papillomavirus prevalence in Iranian women with normal cytology, precancerous cervical lesions and invasive cervical cancer: Implications for screening and vaccination. *Journal of medical virology*, *87*(2), 287-295.

Joura, E. A., Leodolter, S., Hernandez-Avila, M., Wheeler, C. M., Perez, G., Koutsky, L. A., ... & Paavonen, J. (2007). Efficacy of a quadrivalent prophylactic human papillomavirus (types 6, 11,

16, and 18) L1 virus-like-particle vaccine against high-grade vulval and vaginal lesions: a combined analysis of three randomised clinical trials. *The Lancet*, *369*(9574), 1693-1702.

Koutsky, L. A., Ault, K. A., Wheeler, C. M., Brown, D. R., Barr, E., Alvarez, F. B., ... & Jansen, K. U. (2002). A controlled trial of a human papillomavirus type 16 vaccine. *New England Journal of Medicine*, *347*(21), 1645-1651.

Kovacevic, G., Milosevic, V., Knezevic, P., Knezevic, A., Knezevic, I., Radovanov, J., ... & Stanisic, L. (2019). Prevalence of oncogenic Human papillomavirus and genetic diversity in the L1 gene of HPV16 HPV 18 HPV31 and HPV33 found in women from Vojvodina Province Serbia. *Biologicals*, *58*, 57-63.

Langan, D., Higgins, J. P., Jackson, D., Bowden, J., Veroniki, A. A., Kontopantelis, E., ... & Simmonds, M. (2019). A comparison of heterogeneity variance estimators in simulated random-effects meta-analyses. *Research synthesis methods*, *10*(1), 83-98.

Lehtinen, M., Paavonen, J., Wheeler, C. M., Jaisamrarn, U., Garland, S. M., Castellsagué, X., ... & Dubin, G. (2012). Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *The lancet oncology*, *13*(1), 89-99.

Li, N., Franceschi, S., Howell-Jones, R., Snijders, P. J., & Clifford, G. M. (2011). Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. *International journal of cancer*, *128*(4), 927-935.

Lin, Y., Lin, Z., He, F., Chen, H., Lin, X., Zimet, G. D., ... & Wong, L. P. (2020). HPV vaccination intent and willingness to pay for 2-, 4-, and 9-valent HPV vaccines: a study of adult women aged 27–45 years in China. *Vaccine*, *38*(14), 3021-3030.

Liu, H. (2022). Research progress of HPV vaccine for preventing damage from HPV infection. *Highlights in Science, Engineering and Technology*, *8*, 604-610.

Lorincz, A. T., Reid, R. I. C. H. A. R. D., Jenson, A. B., Greenberg, M. D., Lancaster, W. A. Y. N. E., & Kurman, R. J. (1992). Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. *Obstetrics and gynecology*, *79*(3), 328-337.

Lowy, D. R., & Schiller, J. T. (2012). Reducing HPV-associated cancer globally. *Cancer prevention research*, *5*(1), 18-23.

Maine, D., Hurlburt, S., & Greeson, D. (2011). Cervical cancer prevention in the 21st century: cost is not the only issue. *American journal of public health*, *101*(9), 1549-1555.

Malagón, T., Drolet, M., Boily, M. C., Franco, E. L., Jit, M., Brisson, J., & Brisson, M. (2012). Cross-protective efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. *The Lancet infectious diseases*, *12*(10), 781-789.

Mao, C., Koutsky, L. A., Ault, K. A., Wheeler, C. M., Brown, D. R., Wiley, D. J., ... & Proof of Principle Study Investigators. (2006). Efficacy of human papillomavirus-16 vaccine to prevent cervical intraepithelial neoplasia: a randomized controlled trial. *Obstetrics & Gynecology*, *107*(1), 18-27.

Mays, R. M., Sturm, L. A., & Zimet, G. D. (2004). Parental perspectives on vaccinating children against sexually transmitted infections. *Social science & medicine*, *58*(7), 1405-1413.

Michalczyk, K., Misiek, M., & Chudecka-Głaz, A. (2022). Can Adjuvant HPV Vaccination Be Helpful in the Prevention of Persistent/Recurrent Cervical Dysplasia after Surgical Treatment?—A Literature Review. *Cancers*, *14*(18), 4352.

Naud, P. S., Roteli-Martins, C. M., De Carvalho, N. S., Teixeira, J. C., de Borba, P. C., Sanchez, N., ... & Descamps, D. (2014). Sustained efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine: final analysis of a long-term follow-up study up to 9.4 years post-vaccination. *Human vaccines & immunotherapeutics*, *10*(8), 2147-2162.

Paavonen, J., Jenkins, D., Bosch, F. X., Naud, P., Salmerón, J., Wheeler, C. M., ... & Dubin, G. (2007). Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. *The Lancet*, *369*(9580), 2161-2170.

Pimple, S., Mishra, G., & Shastri, S. (2016). Global strategies for cervical cancer prevention. *Current Opinion in Obstetrics and Gynecology*, *28*(1), 4-10.

Powell, S. E., Hariri, S., Steinau, M., Bauer, H. M., Bennett, N. M., Bloch, K. C., ... & Markowitz, L. E. (2012). Impact of human papillomavirus (HPV) vaccination on HPV 16/18-related prevalence in precancerous cervical lesions. *Vaccine*, *31*(1), 109-113.

Ramakrishnan, S., Partricia, S., & Mathan, G. (2015). Overview of high-risk HPV's 16 and 18 infected cervical cancer: pathogenesis to prevention. *Biomedicine & pharmacotherapy*, *70*, 103-110.

Ramanakumar, A. V., Naud, P., Roteli-Martins, C. M., de Carvalho, N. S., de Borba, P. C., Teixeira, J. C., ... & Franco, E. L. (2016). Incidence and duration of type-specific human papillomavirus infection in high-risk HPV-naïve women: results from the control arm of a phase II HPV-16/18 vaccine trial. *BMJ open*, *6*(8), e011371.

Riethdorf, S., Riethdorf, L., Milde-Langosch, K., Park, T. W., & Löning, T. (2000). Differences in HPV 16-and HPV 18 E6/E7 oncogene expression between in situ and invasive adenocarcinomas of the cervix uteri. *Virchows Archiv*, *437*, 491-500.

Risser, W. L., Bortot, A. T., Benjamins, L. J., Feldmann, J. M., Barratt, M. S., Eissa, M. A., & Risser, J. M. (2005, July). The epidemiology of sexually transmitted infections in adolescents. In *Seminars in Pediatric Infectious Diseases* (Vol. 16, No. 3, pp. 160-167). WB Saunders.

Sasagawa, T., Takagi, H., & Makinoda, S. (2012). Immune responses against human papillomavirus (HPV) infection and evasion of host defense in cervical cancer. *Journal of Infection and Chemotherapy*, *18*(6), 807-815.

Seliger, B., Ritz, U., & Soldano, F. (2006). Molecular mechanisms of HLA class I antigen abnormalities following viral infection and transformation. *International journal of cancer*, *118*(1), 129-138.

Smith, J. S., Lindsay, L., Hoots, B., Keys, J., Franceschi, S., Winer, R., & Clifford, G. M. (2007). Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *International journal of cancer*, *121*(3), 621-632.

Stanley, M. A. (2012). Epithelial cell responses to infection with human papillomavirus. *Clinical microbiology reviews*, *25*(2), 215-222.

Suri, H. (2020). Ethical considerations of conducting systematic reviews in educational research. *Systematic reviews in educational research: Methodology, perspectives and application*, 41-54.

Tenny, S., & Hoffman, M. R. (2017). Odds ratio. StatPearls Publishing.

Van de Velde, N., Boily, M. C., Drolet, M., Franco, E. L., Mayrand, M. H., Kliewer, E. V., ... & Brisson, M. (2012). Population-level impact of the bivalent, quadrivalent, and nonavalent human

papillomavirus vaccines: a model–based analysis. *Journal of the National Cancer Institute*, *104*(22), 1712-1723.

Ventura, C., Luís, Â., Soares, C. P., Venuti, A., Paolini, F., Pereira, L., & Sousa, Â. (2022). The Effectiveness of Therapeutic Vaccines for the Treatment of Cervical Intraepithelial Neoplasia 3: A Systematic Review and Meta-Analysis. *Vaccines*, *10*(9), 1560.

Villa, L. L., Costa, R. L., Petta, C. A., Andrade, R. P., Ault, K. A., Giuliano, A. R., ... & Barr, E. (2005). Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *The lancet oncology*, *6*(5), 271-278.

Weingarten, M. A., Paul, M., & Leibovici, L. (2004). Assessing ethics of trials in systematic reviews. *Bmj*, *328*(7446), 1013-1014.

World Health Organization. (2017). Human papillomavirus vaccines: WHO position paper, May 2017–Recommendations. *Vaccine*, *35*(43), 5753-5755.

Yang, X., Cheng, Y., & Li, C. (2017). The role of TLRs in cervical cancer with HPV infection: a review. *Signal Transduction and Targeted Therapy*, *2*(1), 1-10.