

Clearance of human papillomavirus infection in patients with cervical intraepithelial neoplasia

A systemic review and meta-analysis

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Abstract

Background: There are currently no available standard drugs treating human papillomavirus (HPV) infection, especially for patients with low-grade cervical lesion. Several therapies are explored but the results are inconclusive. The objective of this study was to evaluate the efficacy of reported non-invasive treatments in patients with HPV infection and cervical lesions by meta-analysis.

Methods: A comprehensive search of prospective and randomized studies published from April 2000 to April 2020 was conducted in electronic databases. The statistical analyses of the pooled risk ratios (RRs) and the corresponding 95% confidence intervals (95% CIs) were performed using the Revman 5.2 software.

Results: Twelve articles including 12 randomized controlled studies and 1 prospective controlled randomized pilot study were enrolled. Therapeutic medications included biological and herbal regimen, interferon regimen and probiotics. The meta-analysis showed the experimental treatments had a statistically significant improvement in HPV clearance rate compared with the controls (RR = 0.71, 95% CI [0.63, 0.80], $P < .00001$); subgroup analyses stratified by regimen categories were consistent with results in the overall group. Treatment using biological and herbal regimen, interferon regimen or probiotics also resulted in a beneficial outcome in regression rate of cervical lesions compared with the controls (RR=0.55, 95% CI [0.39, 0.79], $P = .001$). The trend was more favorable in the probiotics than that in the biological and herbal regimen (RR 0.48 vs 0.72).

Conclusion: Treatment of biological and herbal regimen, interferon regimen and probiotics benefit patients who have HPV infection and cervical lesions. Both the clearance of HPV and regression of cervical lesions are significant. More studies with less heterogeneity are needed to draw a concrete conclusion.

Abbreviations: ASCUS = atypical squamous cells of undetermined significance, CIN = cervical intraepithelial neoplasia, HPV = human papillomavirus, HSIL = high-grade squamous intraepithelial lesion, LSIL = low-grade squamous intraepithelial lesion, RCT = randomized controlled trial.

Keywords: cervical lesion, herb, human papillomavirus, interferon, probiotics

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1. Introduction

Cervical cancer is the fourth most common cancer in women worldwide. According to the most recent Globocan report, there were 569,847 new cases and 311,365 deaths in 2018.^[1] Persistent human papillomavirus (HPV) infection, especially the infection of high-risk subtypes, is a confirmed precursor to the development of almost all invasive cervical carcinomas.^[2–4] The recent application of virus-like particle prophylactic HPV vaccination offers an effective primary prevention strategy for squamous cervical cancer.^[5,6] However, prophylactic vaccines are type-specific (mostly 16 and 18) and mostly target HPV negative populations. They are relatively expensive and inaccessible to many domestic and developing regions and countries.^[7,8]

For populations who are positive of HPV but negative of cervical lesion, the chances of self-regression are relatively high, especially if they are under 30 years old, thus close surveillance is recommended.^[9] However, for patients with cervical lesions, there is no effective treatment directed towards clearance of HPV till date. HPV infections can be treated through laser, cryotherapy, conization, and so on. The rationale is to cause inflammatory reactions of the local tissue so that the body will be stimulated to mount an antibody response which shows an antiviral effect.^[10–14] However, the invasive therapy is often indicated for those who have high-grade squamous intra-

epithelial lesion (HSIL) and potential side effects like late abortions and preterm delivery brought by invasive procedures are major concerns.^[15,16] For patients who have HPV infection and low-grade lesions (LSIL), the preferred care would be frequent surveillance till they clear the infection or develop cervical neoplasia.^[17]

Some noninvasive therapies have been investigated in preclinical and clinical trials for the treatment of HPV infection or cervical lesions. It has been reported that probiotics can contribute to HPV clearance via creating a synergistic environment, enhancing adaptive and innate immunity, and directly demonstrating antiviral effect via specific metabolites.^[18] In addition, interferon and leukocyte extract have been reported as a modulator of the immune response, which up-regulate chemotaxis of natural killer cells and macrophages in tissues infected with fungi, viruses, and parasites.^[19] Several *in vitro* studies also suggest some herbal formulations and their metabolites can be cytotoxic to cervical cancer cells by inhibiting the transcription of HPV E6/E7 oncogene. Such biological and herbal regimens include polyphenon E, curcumin, myrtle, and *Phytolacca Americana*.^[20–23] Notably, these formulations can also be found in food and are of low cost. To explore the clinical efficacy of those non-invasive therapies in patients with both HPV infection and cervical lesions, several clinical trials were conducted. However, the regimens studied were diverse and results were inconsistent. In this study, in order to draw a more concrete conclusion, we conducted a meta-analysis of the currently published prospective and randomized trials on non-invasive treatments for patients with both HPV infection and cervical lesions.

2. Materials and methods

2.1. Search strategy

Prospective controlled studies for all possible non-invasive treatments of HPV infections in patients with cervical intraepithelial neoplasia that were published from April 2000 to April 2020 were searched in PubMed, EMBASE, CNKI, OVID, and Cochrane databases. The search terms for the disease included “HPV,” “human papillomavirus,” “cervical lesion,” “atypical squamous cells of undetermined significance (ASCUS),” “LSIL,” “HSIL,” and “cervical intraepithelial neoplasia (CIN).” The search terms for treatment included “non-invasive,” “oral,” “injection,” “herb,” “probiotics,” “interferon” and “leukocyte.” The search strategies for prospective controlled trials included using the corresponding filters for different databases. The search was updated every week until April 20th, 2020. The study is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.^[24] No ethical approval and patient consent are required because all analyses were based on previously published studies. The analysis is not a registered study.

2.2. Inclusion and exclusion criteria

The following criteria determined the eligible studies:

- (1) women should be diagnosed with cervical or vaginal HPV infection regardless of subtypes;
- (2) cervical lesions were confirmed by Pap smear (ASCUS, LSI, and HSIL) or colonoscopy (CIN) in some, if not all of these women;

- (3) experimental treatments were non-invasive, including oral administration and subcutaneous or intramuscular injection;
- (4) control arm used placebo; active controls with proved effective ingredient were allowed if the schemes were significantly different in frequency or dose compared with experimental treatments;
- (5) prospective and randomized trials;
- (6) numbers of patients who were HPV positive or cervical lesion positive before and after treatments should be clearly described.

The exclusion criteria were trials that

- (1) studied women with cervicitis only or cervical cancer;
- (2) used invasive treatments such as conization or laser;
- (3) did not have the full text available;
- (4) failed to report the required numeric results.

2.3. Data extraction and quality assessment

Two authors independently searched and retrieve the literature. The third author would adjudicate the discrepancy if there were any. Data extracted as study characteristics included first author, published year, study type, HPV type, HPV testing methods, cervical lesion type, cervical lesion testing methods, treatment regimen, and matched parameters. The data collected for statistical analysis demonstrated dichotomous outcomes, as presented by numbers of HPV and lesion positive patients before and after treatment in both arms. When different efficacy criteria (such as partial response based on the area of lesion affected) were defined in individual trials, only those who had complete HPV clearance and normal Pap smear or colonoscopic findings (including inflammatory manifestations) would be considered as HPV-free and lesion-free. If different follow-ups were conducted, the outcomes would be collected for the latest follow up.

The modified Jadad scale was used to investigate the methodological quality of included studies. Four aspects of random sequence production, allocation concealment, blinding method, and withdrawals and dropouts were scored. Studies were rated as either low quality or high quality under scores of 1 to 3 and 4 to 7, respectively.^[25]

2.4. Statistical analysis

Heterogeneity assumptions were tested using Higgins' I^2 test and the I^2 value was a quantitative parameter of inconsistency across studies.^[26,27] When the I^2 value was less than 50%, low heterogeneity was considered and a fixed-effects model was applied otherwise a random-effects model was used. The risk ratio (RR) was the efficacy estimate for the pooled data ($RR = \text{risk of event in experimental group} / \text{risk of event in control group} = (SE/NE)/(SC/NC)$).^[28] The presence of publication bias was evaluated by inspecting the asymmetry in funnel plots visually. When the funnel plots indicated visible asymmetry, Egger test was performed to further measure the bias, which was considered as existing when P was less than .05.

Statistical analyses were conducted using Review Manager (Version 5.2; Copenhagen: the Nordic Cochrane Centre, the Cochrane Collaboration, Copenhagen, Denmark) and Stata (Version 14.0; StataCorp LLC, College Station, TX).

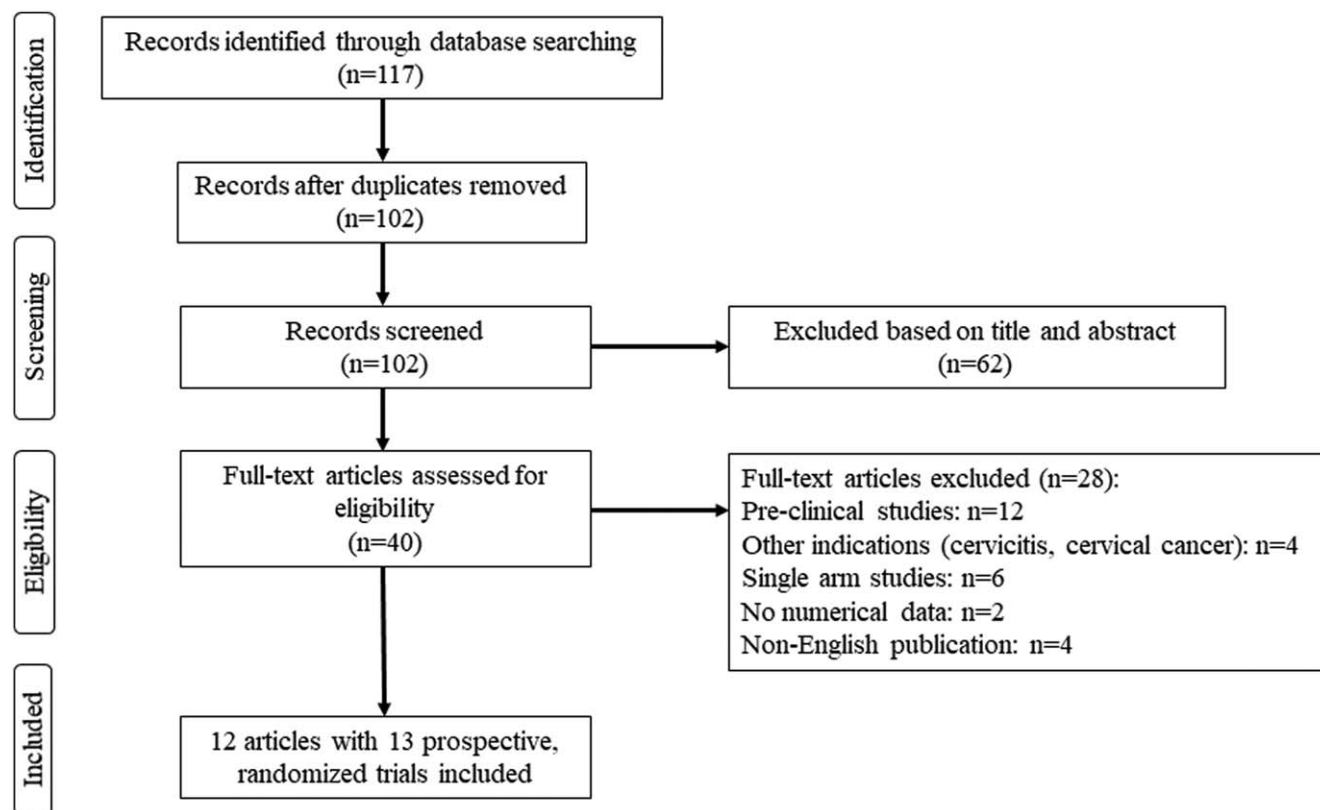


Figure 1. Flow chart of study selection.

3. Results

3.1. Search results

One hundred seventeen studies were preliminarily retrieved according to the developed retrieval strategy. After the removal of duplicates, 102 articles were screened. Sixty-two articles were further excluded based on titles and abstracts. For the remaining 40 articles, 12 were pre-clinical studies; 4 studied other diseases in cervix like cervicitis and cervical cancer; 6 were single arm studies with no controls, 2 did not provide numeric dichotomous outcomes; 4 were non-English publications (Fig. 1). Thus, 12 articles were included in the meta-analysis (Table 1).

3.2. Characteristics of included studies

Among the 12 included articles, 1 study included 2 different treatment regimens and had separate controls for both treatments thus were considered as 2 studies. For the 13 studies, 8 were of high quality (Jadad score of 4–7) and 5 were of low quality (Jadad score of 1–3). Meanwhile, 11 studies were randomized controlled studies (RCTs) and 2 studies were a prospective controlled randomized pilot study. One study used active control (short-term probiotics) while the rest were placebo controlled. Most of the studies did not specify HPV subtypes but 5 studies only included high-risk subtypes of type 16 and 18. Not all patients with HPV infection had cervical lesions. For those who were confirmed as having both HPV infection and cervical lesion, most of lesions were of low grade (ASCUS, LSIL, CIN1) and 2 studies did not mention specific grades by generally describing as

abnormality. Treatments included biological and herbal regimens (polyphenon E, curcumin, basant cream, myrtle, phytolacca americana, and multi-herbal regimens), interferon related regimens (interferon and leukocyte extract), and probiotics. Nine studies listed matched parameters between the experimental and control arms. Most frequently reported matched parameters included age, menopausal status, contraceptive use, smoking status, parity, and sexual activity (Table 1).^[29–40]

3.3. Meta-analysis of efficacy

3.3.1. The clearance of HPV. A total of 12 studies including 548 patients in the experimental arm and 526 patients in the control arm were analyzed. In the experimental arm, 60.22% (330/548) of patients had HPV cleared, while in the control arm, 43.35% (228/526) were HPV negative. Under a fixed-effects model ($I^2=48\%$), the pooled results showed treatments in the experimental arm had a statistically significant difference in HPV clearance compared with the control arm (RR=0.71, 95% CI [0.63, 0.80], $P < .00001$). In summary, treatments of biological and herbal regimen, interferon regimen, and probiotics benefited those patients who had HPV infection (Table 2 and Fig. 2).

Further subgroup analysis explored the different categories of the regimens in clearing HPV infection. For biological and herbal regimen, 7 studies with 619 patients included. After treatments, 31.11% and 47.70% of patients in the experimental and control arms remained HPV positive, respectively. Six out of the 7 studies showed favorable trends (RR <1) for biological and herbal regimen; the further meta-analysis showed that the difference in the clearance rates of HPV between the 2 arms were statistically

Table 1**Characteristics of included studies.**

Author	Year	Country	Study Type	Patient No.	HPV type	HPV testing	Cervical lesion type	Cervical lesion testing	Treatment	Matched parameters	Study quality (Jadad scale)
Acosta-Rios	2017	Mexico	RCT	54	Not known	Not known	CIN 1	Colposcopy	Dialyzable Leukoocyte Extract	Not known	3
Ahn	2003	South Korea	RCT	90	Not known	DNA hybrid capture assay	ASCUS/LSIL/HSIL	Pap smear and colposcopy	Polyphenon E and EGCG	Not known	3
Basu	2013	India	RCT	126	Type 16 or 18	PCR	LSIL	Colposcopy	Basant cream (polyherbal)	Age, parity, menopausal status, HPV type	5
Basu	2013	India	RCT	161	Type 16 or 18	PCR	LSIL	Colposcopy	Curcumin capsule	Age, parity, menopausal status, HPV type	5
Garcia	2014	US	RCT	98	High risk	DNA hybrid capture	CIN1	Colposcopy	Polyphenon E	Age, weight, height, race, ethnicity, smoking status, current sexual activity, age at 1st intercourse, total lifetime partners	6
Gonzalez-Sanchez	2001	Mexico	RCT	122	Not known	Not known	CIN1-3	Pap smear and colposcopy	Interferon beta	Age, beginning of sexual relationships, number of pregnancies, use of contraceptives, types of previous CIN treatments	4
Ijazović	2006	Bosnia	RCT	55	Not known	DNA hybrid capture	ASCUS/LSIL mainly	Pap smear	Interferon and aloe vera	Not known	3
Mutombo	2019	Congo	RCT	59	Not known	PCR	ASCUS and LSIL mainly	Pap smear	AV2 (canvone, eugenol, geraniol, nerolidol)	Age, marital status, education, profession, menopausal status, abortion, miscarriage, lifetime sexual partners, oral contraceptives use, condoms use, use of traditional herbs	6
Nikakhtar	2018	Iran	RCT	60	Not known	DNA hybrid capture assay	Lesion not specified	Colposcopy	Myrtle	Age, marriage duration, parity, detection time of disease, HPV typing, mean affected area, external genital wart, vaginal itching and burning	5
Ou	2019	China	RCT	121	High risk	DNA hybrid capture assay	ASCUS/LSIL	Pap smear	Probiotics	Age, menopausal status, parity, IUD, educational level, history of Th, HR-HPV load, HR-HPV clearance, ASCUS/LSIL	5
Palma	2018	Italy	Prospective controlled pilot study	117	Not known	PCR	Pap smear abnormality	Pap smear and colposcopy	Probiotics	Age, contraceptive methods, regular menses, symptoms prevalence, smoking, multiple partners, bacterial vaginosis, yeast vaginitis, pap smear alterations	3
Verhoeven	2013	Belgium	Prospective controlled pilot study	51	Not known	PCR	LSIL	Pap smear	Probiotics	Age, smoking status, level of stress, alcohol use, hormonal contraception, tampon use, condom use, lifetime partners, cancer history, physical exercise.	3
Yang	2019	China	RCT	79	High risk	DNA hybrid capture	CIN1	Colposcopy	REBACIN (Phytolacca americana)	Not known	4

ASCUS: atypical squamous cells of undetermined significance, CIN = cervical intraepithelial neoplasia, HPV = human papillomavirus, HSIL = high-grade squamous intraepithelial lesion, LSIL = low-grade lesions, RCT = randomized controlled trial.

Table 2
Numeric distributions between experimental arms and control arms for HPV clearance and cervical lesion regression.

Group	Author	Year	Patient No.	Experimental		Control		Experimental		Control	
				HPV+	Total	HPV+	Total	Lesion+	Total	Lesion+	Total
Biological and Herbal	Ahn	2003	79	15	41	21	38	10	40	5	13
	Basu	2013	113	8	65	12	48	3	7	2	2
	Basu	2013	155	14	75	22	80	4	6	0	0
	Garcia	2014	82	31	41	29	41	34	41	35	41
	Mutombo	2019	59	13	27	21	32	6	17	13	24
	Nikakhtar	2018	52	2	27	8	25	16	27	21	25
	Yang	2019	79	15	39	32	40	16	32	31	35
Interferon related	Acosta-Rios	2017	54	Not known	Not known	Not known	Not known	3	27	27	27
	Gonzalez-Sanchez	2001	122	18	61	35	61	Not known	18	Not known	9
	Iljazović	2006	55	25	35	18	20	Not known	35	Not known	20
Probiotics	Ou	2019	121	26	62	27	59	4	13	6	9
	Palma	2018	106	33	51	48	55	8	39	25	40
	Verhoeven	2013	51	18	24	25	27	12	24	19	27

HPV = human papillomavirus.

significant (RR=0.66, 95% CI [0.47, 0.92], P=.01). Only 2 studies described interferon treatment for HPV infection. With heterogeneity observed (I²=76%), the pooled data showed a favorable but insignificant benefit of interferon over the control (RR=0.66, 95% CI [0.39, 1.10], P=.11). Three studies compared probiotics and controls in HPV clearance. Notably, 1 study (Palma et al) used active control, comparing standard treatment plus short-term (3 months) vaginal Lactobacillus implementation versus the same standard treatment plus long-lasting (6 months) vaginal Lactobacillus administration. The meta-analysis of the 278 patients showed no heterogeneity (I²=0%) and statistically significant difference between probiotics and the control (RR=0.81, 95% CI [0.68, 0.96], P=.01) (Table 2 and Fig. 3).

3.3.2. Regression of cervical lesion. Among all the included studies, 11 studies reported the numbers of patients who had cervical lesion before and after treatments. In addition, 1 study had no patients with cervical lesion in the control arm, thus the regression of

lesion would not be estimable. Regardless of regimens, the meta-analysis included 273 patients and 243 patients in the experimental and control arms, respectively. All individual studies reported decreased percentages of patients who were still lesion positive after treatments, with 3 studies showed significant difference between the experimental and the control arm. The pooled results showed that treatment using biological and herbal regimen, interferon regimen or probiotics resulted in a beneficial outcome in regression rate of cervical lesions compared with the controls (RR=0.55, 95% CI [0.39, 0.79], P=.001) (Table 2 and Fig. 4).

With only 1 study reported the dichotomous outcomes among interferon related regimens, subgroup analysis was conducted for biological and herbal regimen and probiotics. Compared with 140 patients in the control arm, 164 patients who were treated with biological and herbal regimens showed a significantly decreased rate of lesion positivity after treatments under a random-effects model (RR=0.72, 95% CI [.54, 0.94], P=.02, I²=55%). Three studies with 152 patients showed the lesion regression rate was 68.42% (52/72) in probiotics arm and

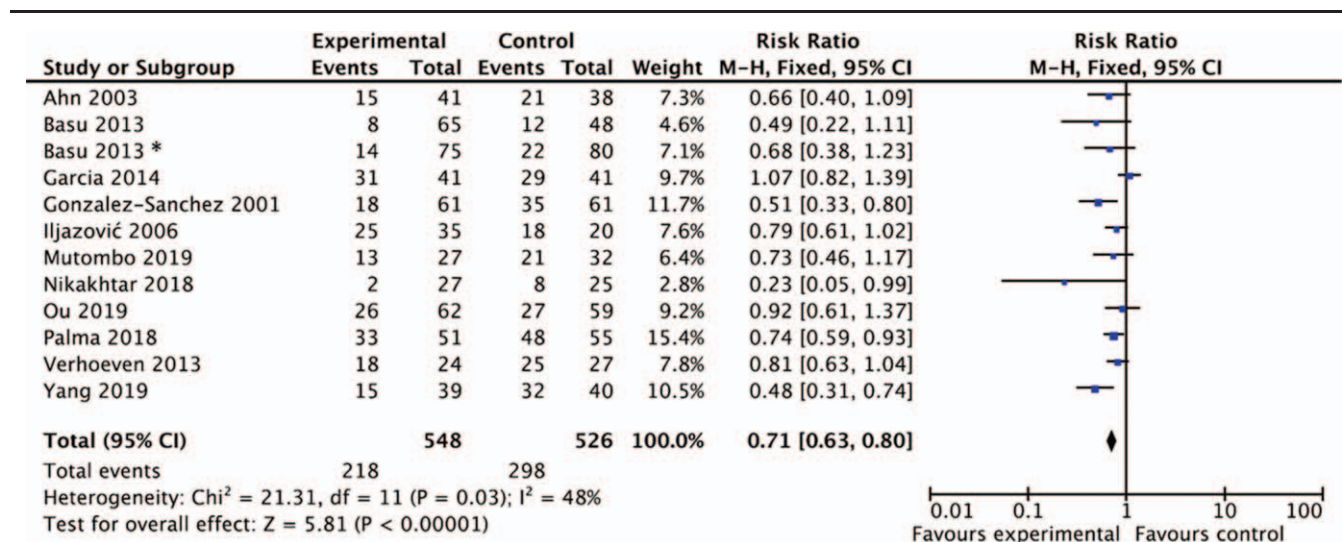


Figure 2. Forest plot of HPV clearance: overall group treatment vs. control. *Basu 2013 studied 2 regimes with separate controls and was considered as 2 trials. HPV = human papillomavirus.

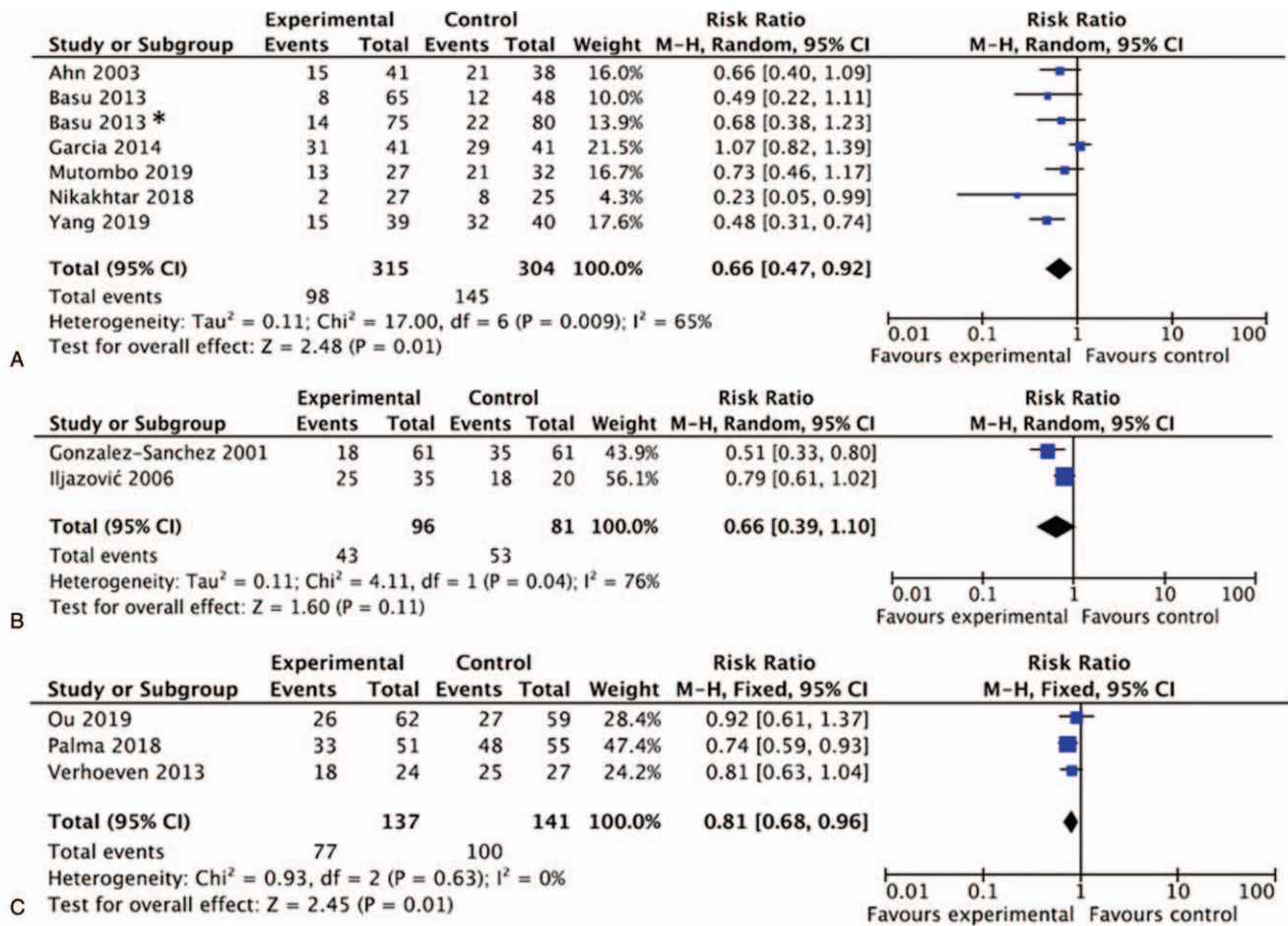


Figure 3. Forest plots of HPV clearance. (A) Biological and herbal regimen vs. control; (B) Interferon vs. control; (C) Probiotics vs. control. *Basu 2013 studied 2 regimes with separate controls and was considered as 2 trials. HPV = human papillomavirus.

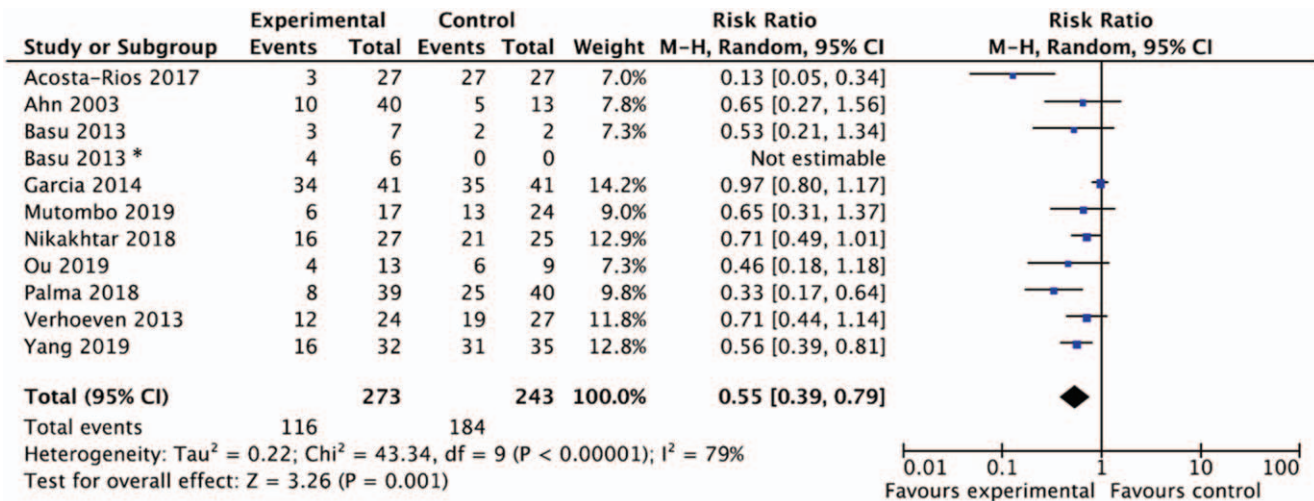


Figure 4. Forest plot of cervical lesion regression: overall group treatment versus control. *Basu 2013 studied 2 regimes with separate controls and was considered as 2 trials.

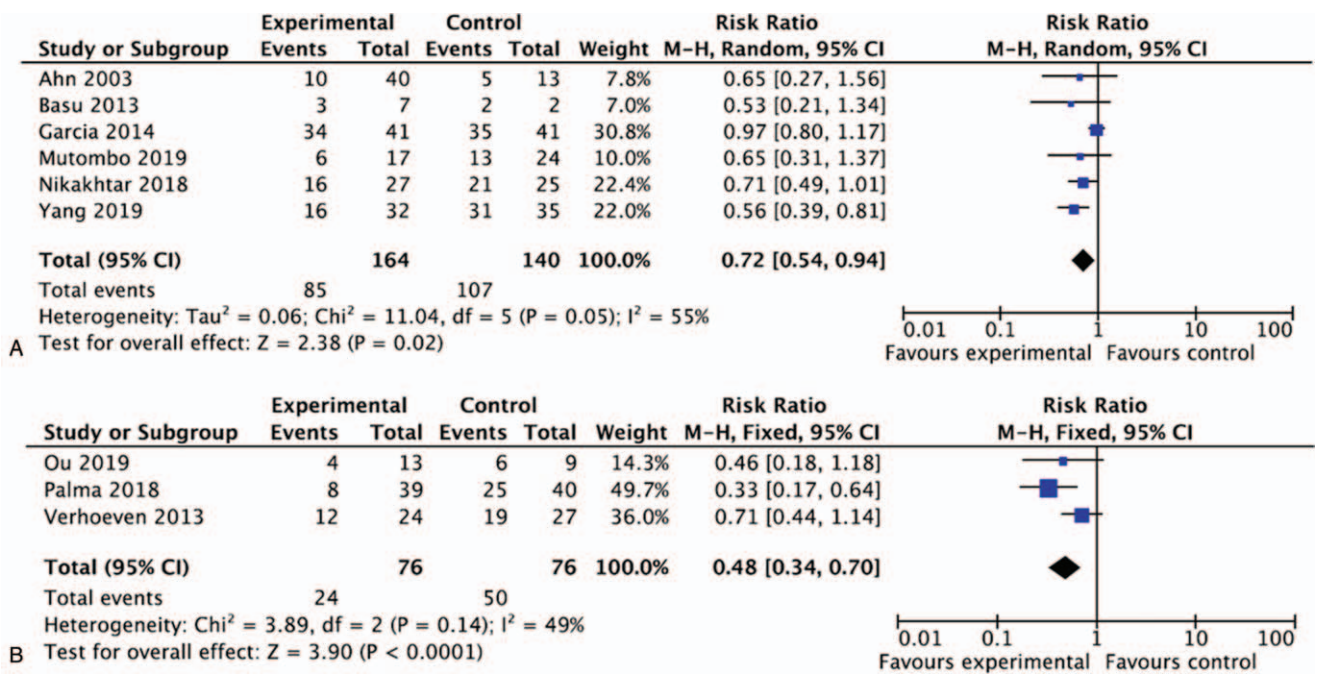


Figure 5. Forest plots of cervical lesion regression. (A) Biological and herbal regimen versus control; (B) Probiotics versus control.

36.11% (26/72) in the control arm, the meta-analysis displayed a significant deference between the 2 arms (RR=0.48, 95% CI ([0.34, 0.70]), P<.0001) and the trend was more favorable compared with the biological and herbal regimen (RR=0.72) (Table 2 and Fig. 5).

3.3.3. Publication bias and sensitivity analysis. The funnel plot demonstrated a symmetrical shape for overall group HPV clearance studies as well as subgroup analysis for probiotics in both HPV clearance and lesion regression, suggesting no significant publication bias. Despite the interferon subgroup analysis with only 2 studies included, the distribution was imbalanced for overall group cervical lesion regression studies

and analysis for biological and herbal subgroups (Fig. 6). Further Egger test also demonstrated significant bias in biological and herbal subgroups (P<.05).

Sensitivity analysis was conducted by checking the consistency of the overall effect estimate using the leave-one-out method. Studies with significantly smaller sample sizes were removed but the value of I² was similar (data not shown). The exclusion of any single study did not materially change the summary result. We believe that the heterogeneity may arise from studies that used biological and herbal regimens, which were complex in nature. Also, different follow-up periods among different studies and self-regression in low-graded patients might also contribute the heterogeneity.^[41,42]

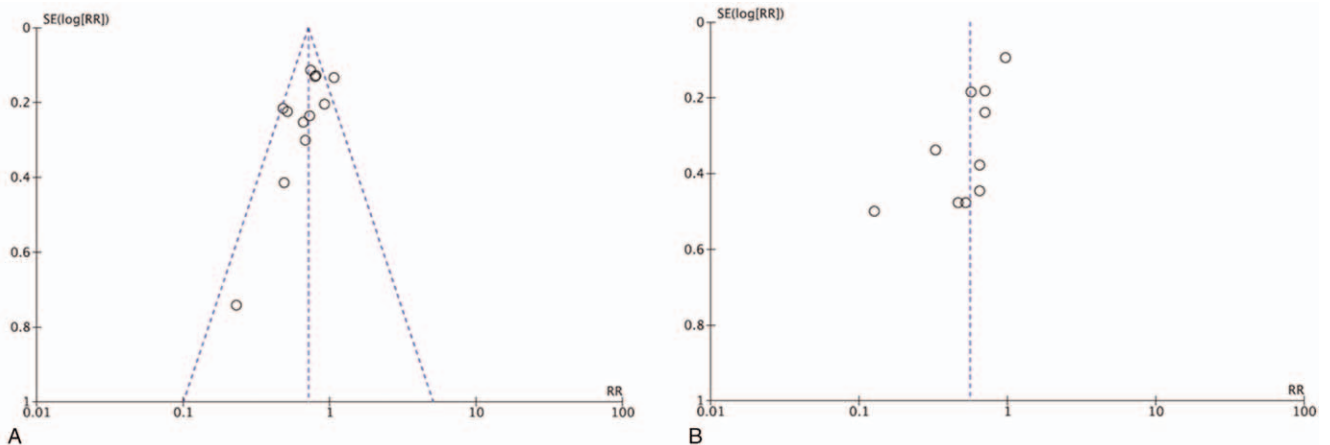


Figure 6. Representative funnel plots. (A) Overall group analysis in HPV clearance; (B) Overall group analysis in cervical lesion regression. HPV = human papillomavirus.

4. Discussion

Cervical malignancies, the commonest infection-associated neoplasm, and its premalignant precursor cervical intraepithelial neoplasia are relative with strains of HPV. Although the virus is highly prevalent, only a few women have a persistent HPV infection and subsequently develop significant disease that requires clinical intervention.^[43] Currently, there is no standard of care for HPV infection, especially for patients with low-grade cervical lesions.

The mechanism of persistent infection was not definite but there is emerging evidence that indicates cervicovaginal microbiota plays an important role in the persistence infection and subsequent disease.^[44–47] The acidic microbial environment in vagina can prohibit pathogen growth through decreasing oxidative stress, modulating immune responses, and expressing bacteriocins.^[48,49] Women with unbalanced composition of the cervicovaginal microbiota, including low *Lactobacillus* abundance and high diversity, are more likely to acquire infections of HIV, HPV, et al. It has been approved in preclinical studies that the presence of certain species of *Lactobacillus* spp. was a protective factor by maintaining the “cervical epithelial barrier” function that prevents entry of HPV to the basal keratinocytes.^[50] Thus, by replenishing the depleted pool of *Lactobacillus* spp., probiotics, which is defined as “live microorganisms that, when administered in adequate amounts, deliver a health benefit on the host” by the World Health Organization, has been suggested recently as an interventional method to promote HPV clearance.^[51] In a recent trial, Palma et al reported that HPV clearance was higher in the treatment of metronidazole plus 6 months vaginal *Lactobacillus* implementation than that with 3 months use.^[38] However, in another RCT conducted by Verhoeven et al, the probiotics of oral *Lactobacillus* on HPV clearance was observed in women with HPV-related LSILs but the result was not significant compared with controls.^[39]

Despite of probiotics, several other non-invasive treatments are also studied in patient with HPV infection and cervical lesions, the most popular ones are biological and herbal regimens which are of low cost and can serve as food adjuvant. For example, polyphenon E, a decaffeinated, enriched, and defined mixture of green tea catechins, was proved in vitro to prohibit the growth of HPV-positive cervical cancer cells in a dose dependent mode.^[52] In a randomized study by Ahn et al, treatment with green tea extracts (polyphenon E; poly E and (–) -epigallocatechin-3-gallate) in 51 patients with HPV infected cervical lesions demonstrated a response rate of 69% (35/51), which was significantly higher as compared with 10% (4/39) in untreated controls.^[30] Another example is myrtle. It is the common name of *M. communis* and is from the family of Myrtaceae. Therapeutic effects of myrtle include antiviral, antibacterial, antioxidant, antifungal, anti-inflammatory, and proapoptotic activities.^[53] Nikakhtar et al published an RCT to compare the efficacy of herbal vaginal suppository (10% of myrtle aqueous extract and 0.5% of myrtle essential oil) and placebo in women with cervicovaginal HPV infection. The interventional group benefited significantly from myrtle, with HPV clearance rate as 92.6% and the change in cervical lesion size as 71.4%.^[36]

For the last decade, intramuscular administration of interferon beta has been used to treat HPV either alone or with other treatments. Both interferon and leukocyte extracts are considered as a modulator of the immune response, which up-regulate the chemotaxis and activation of natural killer cells and macro-

phages. Although the safety profile has been proved acceptable, efficacy results are varied and elucidation especially for patients with both HPV infection and cervical lesions is limited.^[54]

Our study is the first meta-analysis to explore the efficacy of available non-invasive treatments in patients with HPV infection and cervical lesions. The meta-analysis showed the experimental treatments had a statistically significant improvement in HPV clearance rate compared with the controls (RR=0.71, 95% CI [0.63, 0.80], $P < .0001$); subgroup analyses stratified by regimen categories (biological and herbal regimen, interferon regimen or probiotics) were consistent with results in the overall group. Included treatments also resulted in a beneficial outcome in regression rate of cervical lesions compared with the controls (RR=0.55, 95% CI [0.39, 0.79], $P = .001$). The trend was more favorable in the probiotics than that in the biological and herbal regimen (RR 0.48 vs 0.72). Individually, almost all included studies reported a beneficial trend of using experimental treatment but the majority of them displayed an insignificant difference compared with controls. By conducting the meta-analysis, the pooled results showed statistically significant results in both overall group analysis and subgroup analysis (except interferon related regimen in HPV clearance). This proves the importance of this study to draw a more concrete conclusion in those inconclusive trials. In addition, despite the diversity of regimens used in different trials, this study reported not only the overall group results but also subgroup analysis by grouping individual regimens according to anatomical, therapeutic, chemical class. Such analysis would be more interpretable and can potentially facilitate the development of novel medications with similar chemical structure and mechanisms.

Despite our efforts to include all available publications, several limitations to our meta-analysis should not be ignored. First, heterogeneity was noted among individual studies, especially for biological and herbal regimens. Sensitivity analysis using the leave-one-out method presented similar value of I^2 . We believe that the heterogeneity may arise from studies that used biological and herbal regimens, which were complex in nature. Herbal therapy is associated with cultures, while some herbs are popularly used in some countries, they might be never tested in other regions. Also, even though the herbs are under 1 anatomical, therapeutic, chemical class, their mechanisms might differ from each other, thus future analysis is needed to explain the anti-viral effect in a more specific manner.^[55] Meanwhile, different follow-up periods among different studies and self-regression in low-graded patients might also contribute the heterogeneity. Whereas, the exclusion of any single study did not materially change the summary result, the benefit shown in current meta-analysis can still be concluded cautiously. Second, not all of the studies reported matched parameters between experimental and controls. Baseline characteristics such as ethnicity and sexual activity are intrinsic factors known to be significantly associated with microbial stability. For example, it was reported that Caucasian and Asian women displayed a significantly greater prevalence of *Lactobacillus* spp. dominant microbiota, compared to Black and Hispanic women.^[56] Therefore, unreported, and potentially imbalanced factors might lead to an inaccurate explanation of pooled data. Third, although the number of pooled participants was so far the largest, the number of several subgroup analyses was still relatively limited, especially for interferon related regimen. Thus, cautions must be paid when interpreting such subgroup analysis.

5. Conclusion

Treatment of biological and herbal regimen, interferon regimen, and probiotics exhibit beneficial effects in patients who had HPV infection and cervical lesions. However, more studies with less heterogeneity are needed to draw a concrete conclusion.

Author contributions

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