The Significance of noninvasive therapies for cervical infection of high-risk human papilloma virus: A systematic review and meta-analysis

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Research Article

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Abstract

Objective

To assess whether noninvasive therapies were related with clearance of cervical infection of high-risk human papilloma virus (hr-HPV) and regression of mild abnormal cytology related with hr-HPV.

Methods

Until April 2023, we identified a total of 10424 women with cervical infection of hr-HPV and 1966 women with mild abnormal cytology related with hr-HPV from 44 studies meeting the inclusion criteria.

Results

After systematic retrieving literature, we identified 2317 citations, 44 randomized controlled studies were enrolled. Cumulative results suggested noninvasive therapies benefited women who had cervical infection of hr-HPV. Both the clearance of hr-HPV (OR: 3.83, I² = 99%, p < 0.00001) and regression of mild abnormal cytology related with hr-HPV (OR: 3.12, I² = 63%, p < 0.00001) were significant higher than control group. Subgroup analyses stratified by systematic therapy, topical therapy, traditional chinese medicines (TCMs), persistent hr-HPV were consistent with results in the overall group. There was substantial heterogeneity between trials (I² = 87% for clearance of hr-HPV and 63% for regression of cytology), sensitivity analysis was performed by excluding single study one by one, found the cumulative results were stable and dependable. Both the funnel plots for clearance of hr-HPV and regression of abnormal cytology were asymmetrical, significant publication bias might exist.

Conclusion

Noninvasive therapies benefited women who had cervical infection of hr-HPV with/without mild abnormal cytology related with hr-HPV. Both the clearance of hr-HPV and regression of abnormal cytology were significant higher than control group. More studies with less heterogeneity are needed urgently to draw a concrete conclusion.

1. Introduction

Cervical cancer is the most common female genital tract cancer worldwide, it is a major health challenge for women, causes high burden of morbidity and mortality, accounts for nearly 10% of all cancers[1]. Annually, estimated 530,000 new cases are diagnosed worldwide with over 80% during advanced stages[2]. According to the most recent cancer statistics, 2023[3], there are estimated 13,960 new cases and 4,310 deaths in United States. Commonly, it progresses through a sequence of pre-malignant lesions known as cervical intraepithelial neoplasia (CIN) mainly attributed to hr-HPV infection, which is the key etiologic factor to cervical cancer. Fortunately, cervical cancer is the few malignancies with confirmed etiology, maybe prevented by effective treatment for clearing hr-HPV infection.

HPV infection is the most common sexually transmitted infections worldwide, World Health Organization (WHO) estimates that the prevalence of HPV infection is between 9% and 13% of the world population, only in the United States, there are more than 6.2 million women are positive every year[4-6]. Despite the prophylactic vaccines can effectively avoid new infection, are not effective in eradicating existed infection[7]; meanwhile, these vaccines merely induce specific immunity to certain HPV types, the efficacy of cross-immunity for non-covering type is weak[8]; furthermore, their immunization longevity need to be evaluated; finally, the inaccessibility to vaccines in resource-poor regions had already been proven to be responsible for a substantial worldwide burden[9].

Most hr-HPV infections are spontaneous cleared within 6–18 months without interventions[10], they are advised to keep in long-term follow up, this process often causes anxiety and tension among HPV-positive women. Hr-HPV could insert the oncogene fragment into the DNA of the host cells, which makes it more difficult to achieve complete remission, about 10% of women will suffer from persistent hr-HPV infections[11], which is the strongest risk factor for developing CIN and cervical cancer. There are great difficult to predict whether HPV would undergo spontaneous regression or progress, the exact factors associated with clearance or persistent of hr-HPV infections are poorly understood. Currently, there is no cure for persistent hr-HPV infections[12]. Effective intervention for clearing hr-HPV infections has vital significance, maybe helpful to reduce the risk of persistent hr-HPV infections, further reduce the risk of CIN and cervical cancer.

Due to the limited acknowledge of whole biological behavior and molecular characteristics of HPV, difficulties to cultivate HPV in vitro and limited mouse models for HPV-mediated cervical cancer[13]. The intervention of hr-HPV infection has not made significant progress, and the effect is also not satisfactory. There are currently no available standard therapy, especially for patients with/without mild abnormal cytology except following up and monitoring. Conventional invasive methods such as CO2 laser[14] or electrosurgical excision[15] may cause adverse reactions such as stenosis of the cervix, spontaneous abortion and preterm birth. Emerging reports demonstrate noninvasive therapies maybe more acceptable alternative choice for this condition, some noninvasive therapies such as probiotics[16], interferon[17], polyphenon E[18], curcumin[19], myrtle[20], TCMs[21],etc, had been investigated in preclinical and clinical trials, but the results were inconclusive. Interferon (IFN) was the most common reported therapy, exerted antiviral, immuno-regulatory, and anti-tumor effects. Immunologic therapy with IFNs represented a promising anti-HPV modality for clinical, subclinical, and latent disease, however, previous studies reported inconsistent results, the efficacy of IFNs in the treatment of hr-HPV infections had not been established.
Due to lack of consistent and reliable evidence supported, the value of non-invasive intervention for hr-HPV infections was uncertain. High-grade squamous intraepithelial lesion (HSIL) was identified as direct precancerous lesions of cervical cancer, usually invasive surgical treatments such as conization of cervix or loop electrosurgical excision procedure (LEEP), were recommended for those women. For patients who have mild abnormal cytology, including inflammatory cytology, abnormal squamous cells of uncertain significance (ASC-US) and low-grade lesions (LSIL), the preferred therapy was frequent surveillance\(^{22–24}\). Hence, a wait-and-watch approach was usually recommended for positive hr-HPV women with/without mild abnormal cytology to determine whether there is spontaneous regression or progression, such strategy is practicable, but cannot address the anxiety of patients for a long time. In clinical practice, large numbers of positive hr-HPV women seek treatment urgently, several noninvasive therapies are recommended for experimental intervention also, the reported effects were variable and inconsistent, in order to draw a more concrete conclusion, we conducted a meta-analysis of the currently published randomized control trials (RCTs) compared non-invasive treatments to placebo or no treatment for positive hr-HPV women with/without mild abnormal cytology to answer the following questions:

1. whether noninvasive therapies was helpful for clearance of cervical hr-HPV infection?
2. whether noninvasive therapies was helpful for regression of mild abnormal cervical cytology related with hr-HPV infection?
3. whether noninvasive therapies was helpful for clearance of cervical persistent hr-HPV infection?
4. whether systematic, topical and TCMs were effect for positive hr-HPV women with/without mild abnormal cytology?

2. Materials and methods

2.1 Literature search

This systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO; ID: CRD42023418069)\(^{25}\). A detailed protocol was formulated prior to the search including the sources of database, participants, interventions, comparisons, main outcomes and additional outcomes.

Our systematic review was acted in accordance with Preferred Reporting Items for Systematic Reviews and Meta examinations (PRISMA) statement\(^{26}\) (detailed in File S1). A systematical literature search was performed using PubMed, Web of Science, Cochrane library electronic databases, ClinicalTrials, WanFang Data and Chinese National Knowledge Infrastructure (CNKI), the search was performed comprehensively up to April 2023. We sought to identify all researches comparing clearance of hr-HPV and regression of cervical cytology between noninvasive therapies group and control group with no limitation on region or language, control group was limited to placebo or no treatment. We aimed to evaluate the effect of noninvasive treatment by comparing ratio of clearance of hr-HPV and regression of cervical cytology. Literature was queried using the following terms: (Human Papillomavirus Virus or Papillomavirus Virus, Human or Virus, Human Papillomavirus or Human Papillomaviruses or HPV, Human Papillomavirus Virus or Human Papillomaviruses Virus or HPV, Human Papillomavirus Virus or Human Papillomaviruses Virus or HPV, Human Papillomavirus Virus or Human Papillomaviruses Virus or HPV, Human Papillomavirus Virus or Human Papillomaviruses Virus or HPV) and (randomized controlled trial or randomized or placebo) and cervic*.

Related articles of enrolled literature and Google search were used to avoid omitting newly published and internet resources. Clinical studies based on human including RCTs and quasi-RCTs were included without restriction of time. We extracted the following information: the first author, time of study, Study Design, Time of follow up, Research object, number of patients, Treatment method, Clearance of HPV, cytologic regression and adverse event for each enrolled study.

Two reviewers (HY and YZ) evaluated all searched studies independently in order to screen suitable studies according to inclusion and exclusion criteria. The process of selection was conducted on 2 steps. Firstly, we screened titles and abstracts to exclude unmatched studies including in vivo and/or in vitro studies not verified by clinical studies, reviews, case reports, retrospective study, observational study, comments and letters. Secondly, full-text version of remaining studies were obtained and further reviewed according to the following inclusion criteria: a) original studies included hr-HPV infection, b) reported clearance of HPV or cytologic regression, c) compared to placebo or no treatment, and d) full text available. Any differences were resolved by consultation.

2.2 Statistical analysis

Heterogeneity was assessed using Higgins \(^{2}\), which measured the percentage of the total variation across studies due to heterogeneity rather than chance\(^{27}\). \(I^2\) was evaluated as follows: \(I^2 = (Q - df) / Q \times 100\%\), where Q was the Cochran heterogeneity statistic and df was degrees of freedom. \(I^2 < 50\%\), represented insignificant heterogeneity, fix-effects models were adopted to poll ratio of clearance of HPV and cytologic regression; \(I^2 > 50\%\), represented substantial heterogeneity, random-effects models were used to poll research results. Clearance of hr-HPV was calculated on the number of hr-HPV negative at the end of study divided by number of enrolled hr-HPV positive during each group, cytologic regression was calculated on the number of normal cytology at the end of study divided by the number of enrolled mild abnormal cytology during each group.

A funnel plot was generated to identify publication bias, which was a scatter diagram of the hazard ratio (HR) of individual studies on the x axis against the standard error (SE) of the log HR on the y axis. The funnel plot resembled a symmetrical inverter funnel in the absence of publication bias, implied a stable and reliable pooled result; whereas asymmetrical funnel plot represented existed publication bias.

Sensitivity analysis was conducted for all outcome measures to determine whether the conclusions were robust to arbitrary decisions, we performed sensitivity analysis by excluding single studies one by one to evaluate whether the cumulative results changed significantly.

The meta-analysis was performed using the Review Manager 5.3, \(p < 0.05\) was considered statistically significant.
3. Results

3.1. Evidence acquisition

After systematic retrieving literature, we identified 2032 citations related to the concerned subjects, 44 RCTs were enrolled after exclusion of duplicate publications, letters, editorials, reviews not reporting original data and concerned outcomes. The flow chart of evidence acquisition was detailed in Fig. 1. The main characteristics of included studies were summarized in Table 1. Overall, a total of 10424 women with cervical infection of hr-HPV and 1966 women with mild abnormal cytology related with hr-HPV were enrolled, 3870 women accepted non-invasive therapy and 6554 women were enrolled into placebo or watchful waiting group.
### Table 1

Main characteristics of the included studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Design</th>
<th>Time of follow up</th>
<th>Research object</th>
<th>observation group</th>
<th>Treatment method</th>
<th>Clearance of HPV</th>
<th>Cytologic regression</th>
<th>adverse event</th>
<th>Control group</th>
<th>Treatment method</th>
<th>Clearance of HPV</th>
<th>Cytologic regress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Judith A. Smith et al 2022&lt;sup&gt;[28]&lt;/sup&gt;</td>
<td>RCT</td>
<td>6 m</td>
<td>Persistent hr-HPV</td>
<td>AHCC supplementation</td>
<td>14/22</td>
<td>-</td>
<td>4/25</td>
<td>placebo</td>
<td>2/19</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alex Baleka Mutombo et al 2019&lt;sup&gt;[29]&lt;/sup&gt;</td>
<td>RCT</td>
<td>6 m</td>
<td>HPV with VIA</td>
<td>Antiviral AV2</td>
<td>11/17</td>
<td>16/25</td>
<td>similar in 2 groups</td>
<td>placebo</td>
<td>11/31</td>
<td>9/27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joseph E. Tota et al 2020&lt;sup&gt;[30]&lt;/sup&gt;</td>
<td>RCT</td>
<td>12 m</td>
<td>hr-HPV</td>
<td>AS04-HPV-16/18 vaccination</td>
<td>761/1070</td>
<td>-</td>
<td>-</td>
<td>Control Vaccine</td>
<td>1118/1507</td>
<td>-</td>
<td></td>
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<tr>
<td>Luis Serrano et al 2021&lt;sup&gt;[31]&lt;/sup&gt;</td>
<td>RCT</td>
<td>6 m</td>
<td>hr-HPV with LSIL</td>
<td>Papillocare</td>
<td>27/44</td>
<td>39/44</td>
<td>9/44</td>
<td>watchful waiting</td>
<td>11/26</td>
<td>15/26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wenping Wang et al 2022&lt;sup&gt;[32]&lt;/sup&gt;</td>
<td>RCT</td>
<td>6 m</td>
<td>hr-HPV with LSIL</td>
<td>focused ultrasound</td>
<td>72/95</td>
<td>85/95</td>
<td>-</td>
<td>follow up</td>
<td>23/90</td>
<td>51/90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pierre Van Damm et al 2016&lt;sup&gt;[33]&lt;/sup&gt;</td>
<td>RCT</td>
<td>6 m</td>
<td>HPV-16/18</td>
<td>GTL001</td>
<td>4/14</td>
<td>-</td>
<td>-</td>
<td>placebo</td>
<td>3/7</td>
<td>-</td>
<td></td>
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<tr>
<td>Zahra Nikakhtar et al 2018&lt;sup&gt;[20]&lt;/sup&gt;</td>
<td>RCT</td>
<td>2 w</td>
<td>HPV with/without LSIL</td>
<td>myrtle vaginal suppository</td>
<td>25/27</td>
<td>-</td>
<td>-</td>
<td>placebo</td>
<td>17/25</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antonio Gentile et al 2012&lt;sup&gt;[34]&lt;/sup&gt;</td>
<td>RCT</td>
<td>6 m</td>
<td>HPV</td>
<td>PHMB-based solution</td>
<td>45/50</td>
<td>-</td>
<td>-</td>
<td>watchful waiting</td>
<td>35/50</td>
<td>-</td>
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<tr>
<td>Yi Yang et al 2019&lt;sup&gt;[35]&lt;/sup&gt;</td>
<td>RCT</td>
<td>3 m</td>
<td>Persistent hr-HPV</td>
<td>REBACIN</td>
<td>59/95</td>
<td>16/32</td>
<td>no significant</td>
<td>placebo</td>
<td>16/104</td>
<td>4/35</td>
<td></td>
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<tr>
<td>Shu-Guang Zhou et al 2022&lt;sup&gt;[36]&lt;/sup&gt;</td>
<td>RCT</td>
<td>4 m</td>
<td>Persistent hr-HPV</td>
<td>REBACIN</td>
<td>24/35</td>
<td>-</td>
<td>-</td>
<td>watchful waiting</td>
<td>7/35</td>
<td>-</td>
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<tr>
<td>Hyun-Woong Cho et al 2018&lt;sup&gt;[37]&lt;/sup&gt;</td>
<td>RCT</td>
<td>3 m</td>
<td>HPV with LSIL</td>
<td>Oraly-PGA</td>
<td>37/86</td>
<td>42/96</td>
<td>4/96</td>
<td>placebo</td>
<td>20/96</td>
<td>26/99</td>
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<tr>
<td>Zeng xianjing et al 2020&lt;sup&gt;[38]&lt;/sup&gt;</td>
<td>RCT</td>
<td>1 m</td>
<td>hr-HPV</td>
<td>Compound shaji Oil Suppository</td>
<td>47/93</td>
<td>-</td>
<td>-</td>
<td>watchful waiting</td>
<td>27/93</td>
<td>-</td>
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<tr>
<td>Francisco A.R. Garcia et al 2014&lt;sup&gt;[39]&lt;/sup&gt;</td>
<td>RCT</td>
<td>4 m</td>
<td>hr-HPV with LSIL</td>
<td>Oral Polyphenon E</td>
<td>10/41</td>
<td>21/41</td>
<td>well tolerated</td>
<td>placebo</td>
<td>12/41</td>
<td>21/39</td>
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<td>Partha Basu et al 2013&lt;sup&gt;[40]&lt;/sup&gt;</td>
<td>RCT</td>
<td>4 m</td>
<td>hr-HPV</td>
<td>curcumin polyherbal cream</td>
<td>118/140</td>
<td>-</td>
<td>20/140</td>
<td>placebo</td>
<td>94/128</td>
<td>-</td>
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<tr>
<td>Attila Louis Major et al 2021&lt;sup&gt;[41]&lt;/sup&gt;</td>
<td>RCT</td>
<td>6 m</td>
<td>hr-HPV with LSIL</td>
<td>SAM gel</td>
<td>32/65</td>
<td>58/69</td>
<td>No serious AE occurred</td>
<td>placebo</td>
<td>8/76</td>
<td>37/96</td>
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<tr>
<td>Miriam Dellino et al 2022&lt;sup&gt;[42]&lt;/sup&gt;</td>
<td>RCT</td>
<td>12 m</td>
<td>hr-HPV</td>
<td>Oral Lactobacillus</td>
<td>12/80</td>
<td>48/80</td>
<td>-</td>
<td>watchful waiting</td>
<td>7/80</td>
<td>33/80</td>
<td></td>
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<tr>
<td>Bandit Chumworathayi et al 2011&lt;sup&gt;[43]&lt;/sup&gt;</td>
<td>RCT</td>
<td>12 m</td>
<td>hr-HPV with LSIL</td>
<td>Cryotherapy</td>
<td>26/29</td>
<td>-</td>
<td>-</td>
<td>observation</td>
<td>28/31</td>
<td>-</td>
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<td>Liu jiamin et al 2023&lt;sup&gt;[44]&lt;/sup&gt;</td>
<td>RCT</td>
<td>7 m</td>
<td>Persistent hr-HPV</td>
<td>Qingdu Lotion</td>
<td>17/23</td>
<td>7/38</td>
<td>2/38</td>
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<td>2/21</td>
<td>0/21</td>
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<td>Halseh Ayatollahi et al 2022&lt;sup&gt;[45]&lt;/sup&gt;</td>
<td>RCT</td>
<td>3 m</td>
<td>hr-HPV with LSIL</td>
<td>Oral Zinc Sulfate</td>
<td>23/40</td>
<td>21/40</td>
<td>-</td>
<td>watchful waiting</td>
<td>6/40</td>
<td>10/40</td>
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<td>Jin Hwi Kim et al 2011&lt;sup&gt;[46]&lt;/sup&gt;</td>
<td>RCT</td>
<td>3 m</td>
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<td>zinc-citrate compound</td>
<td>46/76</td>
<td>29/56</td>
<td>no significant AEs</td>
<td>watchful waiting</td>
<td>18/118</td>
<td>24/53</td>
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</table>

Abbreviations: RCT = randomized controlled trial, HPV = human papillomavirus, hr-HPV = high-risk human papillomavirus, LSIL = low-grade squamous intraepithelial neoplasia, AE = adverse event.
<table>
<thead>
<tr>
<th>Study</th>
<th>Study Design</th>
<th>Time of follow up</th>
<th>Research object</th>
<th>observation group</th>
<th>Treatment method</th>
<th>Clearance of HPV</th>
<th>cytologic regression</th>
<th>adverse event</th>
<th>Control group</th>
<th>Treatment method</th>
<th>Clearance of HPV</th>
<th>cytologic regress</th>
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<td>RCT</td>
<td>6 m</td>
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<td>Thermal ablation</td>
<td>111/166</td>
<td>72/93</td>
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<td>1171/2716</td>
<td>96/121</td>
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<td>6 m</td>
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<td>6 m</td>
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<td>-</td>
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<td>16/43</td>
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<td>Persistent hr-HPV</td>
<td>ALA-photodynamic therapy</td>
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<td>3/40</td>
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<td>Hildesheim Allan.et al 2007[55]</td>
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<td>6 m</td>
<td>HPV with/without LSIL</td>
<td>Vaginal interferon a2b</td>
<td>31/55</td>
<td>76/83</td>
<td>8/83</td>
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<td>12/29</td>
<td>27/42</td>
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<td>Lin chundan.et al 2021[59]</td>
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<td>hr-HPV with LSIL</td>
<td>scrapping therapy</td>
<td>60/100</td>
<td>53/100</td>
<td>-</td>
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<td>22/50</td>
<td>14/50</td>
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<td>2 Li yunxia.et al 2018[60]</td>
<td>RCT</td>
<td>3 m</td>
<td>HPV</td>
<td>Vaginal interferon</td>
<td>33/60</td>
<td>-</td>
<td>-</td>
<td>watchful waiting</td>
<td>14/60</td>
<td>-</td>
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<td></td>
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<tr>
<td>Chen Yu.et al 2018[61]</td>
<td>RCT</td>
<td>6 m</td>
<td>hr-HPV</td>
<td>Fuzheng Qingdu Granule (herbal)</td>
<td>39/42</td>
<td>-</td>
<td>-</td>
<td>watchful waiting</td>
<td>17/41</td>
<td>-</td>
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<tr>
<td>Li xinchuan.et al 2021[62]</td>
<td>RCT</td>
<td>-</td>
<td>HPV</td>
<td>Baofukang suppository (herbal)</td>
<td>12/28</td>
<td>-</td>
<td>-</td>
<td>watchful waiting</td>
<td>5/18</td>
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<tr>
<td>Shang guangjia.et al 2016[63]</td>
<td>RCT</td>
<td>6 m</td>
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<td>ozone therapy</td>
<td>41/51</td>
<td>-</td>
<td>-</td>
<td>watchful waiting</td>
<td>12/24</td>
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<tr>
<td>Chen xiaojing.et al 2016[64]</td>
<td>RCT</td>
<td>6 m</td>
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<td>69/137</td>
<td>-</td>
<td>No serious AE occurred</td>
<td>watchful waiting</td>
<td>47/132</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td>Hu aiqing.et al 2015[65]</td>
<td>RCT</td>
<td>-</td>
<td>HPV</td>
<td>Vaginal interferon</td>
<td>48/62</td>
<td>-</td>
<td>No serious AE occurred</td>
<td>watchful waiting</td>
<td>4/66</td>
<td>-</td>
<td></td>
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<tr>
<td>Huang weifeng.et al 2014[66]</td>
<td>RCT</td>
<td>2 w</td>
<td>HPV</td>
<td>Gongjingkang gel (herbal)</td>
<td>14/50</td>
<td>-</td>
<td>-</td>
<td>watchful waiting</td>
<td>0/30</td>
<td>-</td>
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</table>

Abbreviations: RCT = randomized controlled trial, HPV = human papillomavirus, hr-HPV = high-risk human papillomavirus, LSIL = low-grade squamous intraepithelial neoplasia (VaIN), AEs = adverse events.
result was stable and dependable.

4.5. Sensitivity analysis was conducted by excluding single studies one by one, found the cumulative results did not change significantly, meant the cumulative
result was stable and dependable.

4.6. Overall, we identified a total of 10424 women with cervical infection of hr-HPV and 1966 women with mild abnormal cytology related with hr-HPV from 44
RCTs met the inclusion criteria, in 2 trials (Huang weifeng.et al 2014[66] and Li yunxia.et al 2018[69]), the treatment group included 2 different therapies, was
artificially divided into 2 trials for further analysis. 44 trials compared the clearance of hr-HPV between non-invasive therapy group and control group, 21 trials
compared the regression of mild abnormal cytology between non-invasive therapy group and control group, 13 trials evaluated the effect of systematic
therapy, 31 trials evaluated the effect of topical therapy, 14 trials evaluated the effect of TCMs. The main characteristics of the enrolled studies was detailed in
Table 1.

3.2. Quality of evidence

The risk of bias of each included RCTs was assessed with the Cochrane Library tool, the results of bias from the included studies were summarized in Figs. 2
and 3. In general, all the trials satisfied the seven domains of bias defined by Cochrane Collaboration. All the included trials claimed to be randomized with
detailed the randomization process except two trials, so were classified as low risk. Meanwhile, most trials were classified as low risk in allocation
concealment, incomplete reporting and selective reporting, due to several trials was not placebo control, blinding of participants and personnel, blinding of
outcome assessment were classified as high risk. Other potential sources of bias were found such as financial support. It is important to note that the
assessment of publication bias was a subjective task since it was based on the personal judgments of the review authors.

3.3. Evidence synthesis

Overall, we identified a total of 10424 women with cervical infection of hr-HPV and 1966 women with mild abnormal cytology related with hr-HPV from 44
RCTs met the inclusion criteria, in 2 trials (Huang weifeng.et al 2014[66] and Li yunxia.et al 2018[69]), the treatment group included 2 different therapies, was
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therapy, 31 trials evaluated the effect of topical therapy, 14 trials evaluated the effect of TCMs. The main characteristics of the enrolled studies was detailed in
table 1.

3.4. Effect of non-invasive therapy for cervical infection of hr-HPV

By polling results together, we observed noninvasive therapy was helpful for clearance of cervical hr-HPV infection (OR:3.83 (95%CI:2.77 ~ 5.30), I² = 87%, test
for overall effect, Z = 8.13; p < 0.0001) (Fig. 4A), the funnel plot for enrolled studies was asymmetrical, significant publication bias could not be
excluded(Fig. 4B). Sensitivity analysis was conducted by excluding single studies one by one, found the cumulative results did not change significantly, meant
the cumulative result was stable and dependable.

3.5. Effect of non-invasive therapy for mild abnormal cytology related with hr-HPV

By polling results together, we observed noninvasive therapy was helpful for regression of abnormal cytology related with hr-HPV (OR:3.12 (95%CI:2.12 ~
4.57), I² = 63%, test for overall effect, Z = 5.81; p < 0.0001) (Fig. 5A), the funnel plot for enrolled studies was asymmetrical, significant publication bias could
not be excluded(Fig. 5B). Sensitivity analysis was conducted by excluding single studies one by one, found the cumulative results did not change significantly, meant
the cumulative result was stable and dependable.

3.6. Effect of systematic noninvasive therapy for cervical infection of hr-HPV with/without mild abnormal cytology

By polling results together, we observed systematic noninvasive therapy was helpful for clearance of hr-HPV infection (OR:1.98 (95%CI:1.22 ~ 3.21), I² = 84%,
test for overall effect, Z = 2.77; p = 0.006) (Fig. 6A) and regression of abnormal cytology related with hr-HPV (OR:2.30 (95%CI:1.70 ~ 3.11), I² = 20%, test for
overall effect, Z = 5.39; p < 0.0001) (Fig. 6B), the funnel plot for enrolled studies was asymmetrical, significant publication bias could not be excluded (Fig. 6C,
D). Sensitivity analysis was conducted by excluding single studies one by one, found the cumulative results did not change significantly, meant the cumulative
result was stable and dependable.
3.7. Effect of topical noninvasive therapy for cervical infection of hr-HPV with/without mild abnormal cytology

By polling results together, we observed topical noninvasive therapy was helpful for clearance of hr-HPV infection (OR:4.78 (95%CI:3.58 ~ 6.37), $I^2 = 65\%$, test for overall effect, $Z = 10.65$, $p < 0.00001$) (Fig. 7A) and regression of abnormal cytology related with hr-HPV (OR:3.76 (95%CI:2.05 ~ 6.91), $I^2 = 70\%$, test for overall effect, $Z = 4.26$, $p < 0.0001$) (Fig. 7B), the funnel plot for enrolled studies was asymmetrical, significant publication bias could not be excluded (Fig. 7C, D). Sensitivity analysis was conducted by excluding single studies one by one, found the cumulative results did not change significantly, meant the cumulative result was stable and dependable.

3.8. Effect of traditional chinese medicines for cervical infection of hr-HPV with/without mild abnormal cytology

By polling results together, we observed traditional chinese medicines was helpful for clearance of hr-HPV infection (OR:3.83 (95%CI:2.32 ~ 6.33), $I^2 = 57\%$, test for overall effect, $Z = 5.24$, $p < 0.00001$) (Fig. 8A) and regression of abnormal cytology related with hr-HPV (OR:2.53 (95%CI:1.59 ~ 4.03), $I^2 = 50\%$, test for overall effect, $Z = 3.93$, $p < 0.0001$) (Fig. 8B), the funnel plot for enrolled studies was asymmetrical, significant publication bias could not be excluded (Fig. 8C, D). Sensitivity analysis was conducted by excluding single studies one by one, found the cumulative results did not change significantly, meant the cumulative result was stable and dependable.

3.9. Effect of non-invasive therapy for cervical persistent infection of hr-HPV

By polling results together, we observed non-invasive therapies was helpful for clearance of persistent hr-HPV infection (OR:9.66 (95%CI:6.11 ~ 15.27), $I^2 = 0\%$, test for overall effect, $Z = 9.70$, $p < 0.00001$) (Fig. 9A), the funnel plot for enrolled studies was asymmetrical, significant publication bias could not be excluded (Fig. 9B). Sensitivity analysis was conducted by excluding single studies one by one, found the cumulative results did not change significantly, meant the cumulative result was stable and dependable.

4. Discussion

The present study systematically reviewed the existed evidences about the non-invasive therapies for cervical infection of hr-HPV, focused on the clearance of hr-HPV and regression of mild abnormal cytology related with hr-HPV, thus reported notable results. First, we reported polled outcomes of the largest amount (10424 women with cervical infection of hr-HPV and 1966 women with mild abnormal cytology related with hr-HPV) up to now, found there were significant different ratio of hr-HPV clearance and abnormal cytology regression. Second, there was no cue for persistent hr-HPV infections until now, which was the strongest risk factor for CIN and cervical cancer, we found non-invasive therapies was helpful for clearance of persistent hr-HPV infection, provided a new strategy and direction for clinical treatment of hr-HPV and prevention of cervical cancer. Third, subgroup analysis found systematic therapy, topical therapy and TCMs had consistent results, further verified the significance of non-invasive therapies, positive non-invasive treatment might be reasonable alternative strategy for passive frequent surveillance.

HPV was double-stranded DNA (dsDNA) viruses belonging to the papillomavirus family, according to the carcinogenic ability, it was divided into low risk HPV(type of 6, 11, 42, 43, etc) and high risk HPV(type of 16, 18, 31, 33, etc)[71]. They were mainly transmitted by sexual behavior and particularly frequent in sexual active women. Mostly, the infection regressed autonomously within 2 years, left about 10% persistent infection might progress to dysplastic lesions from low to high grade intra-epithelial neoplasia, a few might progress to cervical cancer. Therefore, effective therapies for clearance of hr-HPV might significantly decrease the risk of persistent infection of hr-HPV, precancerous neoplasia and cervical cancer.

Due to high ratio of auto-regression and uncertain effect of current intervention for hr-HPV, close surveillance was recommended for women with hr-HPV infection, even for those with mild abnormal cytology[72]. However, simply wait-and-watch approach caused serious psychological burden to hr-HPV positive women for a long time, large numbers of patients and medical staff sought effective treatment urgently. several non-invasive therapies such as CO₂ laser[73], cryotherapy[74], photodynamic therapy[75], multiple medicines[76–78] and so on, were clinical tried for decades, the reported effects were variable and inconsistent, the significance of non-invasive therapies for hr-HPV infection was controversial. The objective of this study was to evaluate the effect of reported non-invasive therapies by meta-analysis.

At first, we evaluated the effect of non-invasive therapy for clearance of cervical hr-HPV infection, after polling the results of existed 44 RCTs concerned the topic, we observed the clearance of hr-HPV in experimental group was significant higher than control group (OR:3.83 (95%CI:2.77 ~ 5.30), $p < 0.0001$), meant noninvasive therapy was helpful for clearance of cervical hr-HPV infection, sensitivity analysis found the cumulative result was stable and dependable. Several non-invasive therapies have been investigated for the treatment of HPV infection for decades. It had been reported that the oral use of probiotics might modify the microbiota of inferior reproductive tract and increase HPV clearance[79], interferon[80] and leukocyte extract[81] had been reported as immunomodulator to induce humoral and cellular immunity against HPV, several in vitro studies also suggested some TCMs and their extract including baofukang suppository[82], polyphenon E[83], curcumin[84], and myrtle[85] could inhibit the transcription of HPV E6/E7 oncogene and replication of HPV, several therapeutic vaccines had been reported favor of HPV clearance. However, the results based on RCTs was inconsistent, Bandit Chumworathayi.et al[86] compared the clearance rate of HPV infection among women aged older than 30 years with biopsy-confirmed LSIL after cryotherapy, found the difference in the HPV clearance rate between two groups was inconclusive (89.7% during cryotherapy group vs 90.3% during observed group), Yu-Che Ou.et al[87] and Veronique Verhoeven.et al[88] found oral probiotic did not influence genital hr-HPV clearance, Miriam Dellino.et al[89] found oral Lactobacillus crispatus did not
influence genital hr-HPV clearance, Luis Serrano et al compared efficacy of a Coriolus versicolor-Based Vaginal Gel (Papilocare) for hr-HPV infection, demonstrated a better clearance of hr-HPV (59.6% during Papilocare group vs 41.9% during observed group), Wenping Wang et al evaluated the efficacy of focused ultrasound (FU) for hr-HPV infection with LSL, found FU might eliminate hr-HPV

infection with few adverse effects and good tolerance, with a clearance rate of 75.6% in FU group compared to 25.6% in observed group. These contradict results made the significance of non-invasive therapy for cervical hr-HPV infection was not defined yet. Some researchers suggested that asymptomatic HPV infection should not be treated until complication with precancerous lesions, as a result, hundreds of un-treated HPV-positive patients lived in a state of anxiety and terror for years. Present meta-analysis showed the non-invasive treatments had a statistically significant improvement in HPV clearance rate compared with the controls. Active treatment with simple, economic, effective and non-invasive approaches to promote HPV clearance might be a reasonable alternative for surveillance.

Cytology combined with hr-HPV testing remained the mainstay of cervical cancer prevention, especially in developing countries, abnormal cytology was a sign that required close attention and positive treatment. The multisteps progression from ASCUS to cervical intraepithelial neoplasia (CIN), further to cervical cancer represented loss of control of HPV duplication and atypical transformation of cervical epithelial cells. High-grade CIN was commonly treated by surgical excisional procedures such as cold knife conisation and loop electrosurgical excision procedure (LEEP), the excised cervical tissue required further pathological evaluation to rule out incomplete excision and complicating cervical cancer. However, surgery carried the risk for pregnancy associated complications, meanwhile, might not effective clear HPV and carry a higher risk of recurrence. Usually, mild abnormal cytology related with HPV did not need excisional procedures because of high ratio of spontaneous regression rate and lower risk of cervical cancer, however, about 7% of CIN1 lesions might progress to high-grade CIN within 1 year and required surgical treatment. Effective non-surgical therapies were needed urgently for these patients to reduce the rate of progression. Present meta-analysis showed the non-invasive treatments had a statistically benefit for regression of mild abnormal cytology related with hr-HPV compared with the controls, non-invasive therapy might be a suitable treatment for these patients.

Several treatment strategies had been reported for hr-HPV, including ablation, cytotoxic agents, photodynamic therapy, and medical immunomodulators. Multiple medicines were reported in clinical trials, such as AV2, 5-Fluorouracil, imiquimod, cidofovir, retinoic acid, beta and alpha-Interferon, probiotics and so on, with different outcomes. During present meta-analysis, we also found significant heterogeneity among individual studies, might affect the reliability of the polled results. So, a meta-regression based on types of treatment was adopted to verify the results, types of treatment was divided into systematic therapy, topical therapy and traditional chinese medicines, subgroup analysis also reached consistent results, meanwhile the heterogeneity was obviously reduced, further confirmed the effectiveness of the non-invasive therapies.

Hr-HPV could integrate their oncogenic DNA into the host genome and persist in the cervical epithelium for years or even decades, most hr-HPV infections were spontaneous cleared within 6–18 months without interventions, however, about 10% of women might suffer from persistent hr-HPV infections, which was the strongest risk factor for cervical cancer and precancerous lesions. There was currently no standard therapy for persistent hr-HPV infections yet. To evaluate whether noninvasive therapy was helpful for clearance of cervical persistent hr-HPV infection, further subgroup analysis was implemented, we identified 5 RCTs with no heterogeneity, enrolled 430 patients, compared the effect of non-invasive therapy to placebo/surveillance, found the ratio of persistent hr-HPV clearance in experimental group was 9.66 times higher than control group, suggested non-invasive therapy was effective for persistent hr-HPV infections.

The main purpose of performing a meta-analysis was to try to provide more convincing evidence to answer the key clinical issue than single primary study, the key clinical issue we focused was whether noninvasive therapy was related with clearance of cervical hr-HPV infection and regression of mild abnormal cytology related with hr-HPV. Present meta-analysis enrolled well designed RCTs to provide high-level evidence. However, there were various therapies were studied, the reported effects were variable, our polled results suggested non-invasive therapy was effective, which therapy was more effective than others could not be concluded, because we enrolled only RCTs compared to placebo or no treatment. This was the the major limit of present meta-analysis. Further meta-analysis compared the effect between various therapies will provide better reference for clinical treatment. Second, the duration of follow up was too short, less than one years in vast majority of RCTs, shorter than the reported most common spontaneous clearance period by previous literature, the insufficient observation time might affect the judgment of therapeutic effect. Third, we tried to compared the adverse events between experimental group and control group to evaluate the safety of non-invasive therapies, unfortunately, only a few RCTs detailedly reported the adverse events, however, the reported types, severity, incidence and statistical methods of adverse events lacked standardized statistics, we could not quantitatively synthesize and compare the adverse events between experimental group and control group, we could not draw conclusive conclusion about the safety, according to the description during these trials, no serious adverse events had been reported, we could speculate the non-invasive therapies were well tolerated. At last, we must state briefly, although sensitivity analysis found the cumulative results were stable and dependable, there were significant heterogeneity among individual studies, even during meta-regression based on types of treatment, meanwhile the funnel plot for enrolled studies was asymmetrical, significant publication bias could not be excluded, due to these limitation, caution must be paid when interpreting such results.

The main strengths of our review and meta-analysis were scientifically incorporating the results of existed RCTs, the collection of the largest amount of HPV positive women until now, comprehensive assessment all of available treatments and focusing on both the clearance of HPV and the regression of abnormal cytology related with HPV. 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 regimens into systematic therapy, topical therapy and TCMs, such analysis would be more interpretable and could potentially facilitate the development of novel medications with similar mechanisms.
5. Conclusions

Noninvasive therapy benefited women who had cervical infection of hr-HPV with/without mild abnormal cytology related with hr-HPV. Both the clearance of hr-HPV and regression of abnormal cytology were significant. However, more studies with less heterogeneity are needed to draw a concrete conclusion.

Declarations

Author contributions

HY and YZ were major contributor in writing the manuscript and retrieving the literature. All authors read and approved the final manuscript.

Ethics approval and Consent to participate

Ethics statement and consent to participate statement are not applicable because this study is based exclusively on published literature.

Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Acknowledgment

Not applicable.

Funding

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References


Figures

**PRISMA 2009 Flow Diagram**

- **Identification**
  - Records identified through database searching (n = 2271)
  - Additional records identified through other sources (n = 46)

- **Screening**
  - Records after duplicates removed (n = 654)
  - Records screened (n = 654)
  - Records excluded (n = 463)

- **Eligibility**
  - Full-text articles assessed for eligibility (n = 191)
  - Full-text articles excluded, with reasons (n = 147)

- **Included**
  - Studies included in qualitative synthesis (n = 44)
  - Studies included in quantitative synthesis (meta-analysis) (n = 44)

**Figure 1**

Flow diagram for evidence acquisition in the systematic review and meta-analysis
Figure 2

Risk of bias graph: review author's judgments about each risk of bias item presented as percentages across all included studies.

Figure 3

Risk of bias graph: review author's judgments about each risk of bias item presented as percentages across all included studies.
Risk of bias summary: review author’s judgments about each risk of bias item for each included study

Figure 4

Effect of non-invasive therapies for cervical infection of hr-HPV. A). Forest plot of non-invasive therapies for cervical infection of hr-HPV. B). Funnel plot of non-invasive therapies for cervical infection of hr-HPV.
Figure 5

Effect of non-invasive therapies for mild abnormal cytology rated with hr-HPV. A). Forest plot of non-invasive therapies for mild abnormal cytology rated with hr-HPV. B). Funnel plot of non-invasive therapies for mild abnormal cytology rated with hr-HPV.
Figure 6

Effect of systematic non-invasive therapies for hr-HPV. A). Forest plot of systematic non-invasive therapies for cervical infection of hr-HPV. B). Forest plot of systematic non-invasive therapies for mild abnormal cytology rated with hr-HPV. C). Funnel plot of systematic non-invasive therapies for cervical infection of hr-HPV. D). Funnel plot of systematic non-invasive therapies for mild abnormal cytology rated with hr-HPV
Figure 7

Effect of topical non-invasive therapies for hr-HPV. A). Forest plot of topical non-invasive therapies for cervical infection of hr-HPV. B). Forest plot of topical non-invasive therapies for mild abnormal cytology rated with hr-HPV. C). Funnel plot of topical non-invasive therapies for cervical infection of hr-HPV. D). Funnel plot of topical non-invasive therapies for mild abnormal cytology rated with hr-HPV
Figure 8

Effect of TCMs for hr-HPV. A). Forest plot of TCMs for cervical infection of hr-HPV. B). Forest plot of TCMs for mild abnormal cytology rated with hr-HPV. C). Funnel plot of TCMs for cervical infection of hr-HPV. D). Funnel plot of TCMs for mild abnormal cytology rated with hr-HPV.
Figure 9

Effect of non-invasive therapies for persistent infection of hr-HPV. A). Forest plot of non-invasive therapies for persistent infection of hr-HPV. B). Funnel plot of non-invasive therapies for persistent infection of hr-HPV.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PRISMA2020checklist.docx