Chlamydia Trachomatis Infection-Associated Risk of Cervical Cancer

A Meta-Analysis

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Abstract: As whether Chlamydia trachomatis infection increases the risk of cervical cancer is controversial in the literature, we performed a meta-analysis.

Based on a comprehensive search of publications in the Medline, Cochrane, and EMBASE databases, we identified and extracted data from all relevant articles examining C. trachomatis infection and the risk of cervical cancer. The quality of each included study was assessed according to the 9-star Newcastle–Ottawa scale. The strength of association between the C. trachomatis and risk of cervical cancer was estimated by odds ratio (OR) and 95% confidence intervals (CIs). This review was registered at PROSPERO with registration No. CRD42014015672.

A total of 22 studies with 4291 cervical cancer cases and 7628 controls were identified. Overall, C. trachomatis was significantly linked to increased cervical cancer risk in prospective studies (OR = 2.21, 95% CI: 1.88–2.61, P < 0.001), as well as in retrospective studies (OR = 2.19, 95% CI: 1.74–2.74, P < 0.001). Additionally, with a multivariate logistic regression analysis adjusted for HPV and age, C. trachomatis infection was identified as an independent predictor of cervical cancer in 11 studies (OR = 1.76, 95% CI: 1.03–3.01, P = 0.04). Coinfection of human papilloma virus and C. trachomatis has a higher risk of cervical cancer (OR = 4.03, 95% CI: 3.15–5.16, P < 0.001). A subgroup analysis based on histological type indicated an elevated risk for both squamous cell carcinoma (OR = 2.21, 95% CI: 2.00–2.45, P < 0.001), and adenocarcinoma (OR = 1.61, 95% CI: 1.21–2.15, P = 0.001), in association with C. trachomatis. Subgroup analysis by where C. trachomatis infection was detected showed a significantly higher risk of cervical cancer associated with C. trachomatis infection detected in serum (OR = 2.20, 95% CI: 2.01–2.42, P < 0.001), cervical tissue blocks (OR = 2.88, 95% CI: 1.21–6.83, P = 0.02), and cervical secretion (OR = 2.71, 95% CI: 1.41–5.20, P = 0.003), especially in serum with no obvious heterogeneity.

In conclusion, our novel data demonstrate that individuals infected with C. trachomatis have a higher risk of cervical cancer. Therefore, it is necessary to expand C. trachomatis infection screening and treat women with C. trachomatis promptly, particularly those with human papillomavirus infections. This approach will not only protect against pelvic inflammatory disease and infertility, but may also prevent cervical cancer.

INTRODUCTION

Cervical cancer, the 3rd most frequent cancer and the 4th leading cause of cancer death among women worldwide, accounts for nearly 10% of the total newly diagnosed cancer cases and 8% of the total cancer deaths.1 Human papillomavirus (HPV) is now considered the principal etiological agent in cervical cancer.2 However, HPV infection is mostly transient and only a small percentage of females with persistent infection eventually develop cervical cancer.2 Therefore, there may be other cofactors involved in enhancing the susceptibility to cervical cancer after HPV infection by facilitating HPV persistence. Behavioral and lifestyle factors and sexually transmitted infections such as bacterial vaginosis, Chlamydia trachomatis (C. trachomatis), herpes simplex virus, and human immunodeficiency virus have been identified as possible cofactors involved in cervical carcinogenesis.3 C. trachomatis is one of the most common sexually transmitted pathogens in women. Epidemiological studies have shown a higher rate of C. trachomatis infection in patients with cervical cancer.4 In contrary, other researches have failed to find any association between infection with C. trachomatis and cervical cancer.5–8 For example, Tungsritrong et al9 in a nested case–control study in North-East Thailand indicated lack of significant effects of C. trachomatis infection on cervical cancer risk. Therefore, the question of whether C. trachomatis infection increases the risk of cervical cancer has so far not been answered and is still a matter of debate. Hence, we conducted a meta-analysis on all eligible case–control studies, cross-sectional studies, and cohort studies to systematically examine the association between C. trachomatis infection and cervical cancer risk. To our knowledge this is the 1st meta-analysis which provides comprehensive and quantitative evidence of the association between C. trachomatis infection and cervical cancer risk.
METHODS

Data Sources and Search Strategy

In accordance with the PRISMA guidelines, we identified published studies through a systematic review of Medline (via PubMed), Cochrane database, and EMBASE (via Ovid) from the inception to June 31, 2015, with the following search terms: ("Chlamydia trachomatis") AND ("cervical carcinoma OR cervical cancer OR cancer of the cervix OR carcinoma of the cervix OR cervical neoplasm OR cervical dysplasia OR cervical intraepithelial neoplasia"). We also checked reference lists and citation histories during the search.

The following inclusion criteria were used in the meta-analysis: case–control study, cross-sectional study, or cohort study on the association between C. trachomatis and cervical cancers risk; the diagnosis of cervical cancer was confirmed histopathologically; sufficient sample size for estimating an odds ratio (OR) with 95% confidence intervals (CIs); studies were published in English; and the meta-analysis was restricted to original articles (no expert opinions, editorials, or reviews). Conference abstracts and other unpublished articles were also excluded. Studies were excluded if they did not meet all criteria. For multiple publications reporting the same cohort study, the largest or most recent publication was used in the meta-analysis. The approval of the study was obtained from the local research ethics committee. The protocol for this study was published on the International Prospective Register of Systematic Reviews, or PROSPERO. The registration number was CRD42014015672.

Data Extraction and Quality Assessment

The data extraction was performed by 2 investigators independently and conflicts were resolved by consensus. For each study, the following data were extracted: first author’s name, year of publication, country of origin, study year (s), study design, age range, duration of follow-up, test method for C. trachomatis, number of cases, and controls, as well as adjusted or crude OR with 95% CI and adjusted variables that entered into the multivariable model as potential confounders. Based on the extracted data, the quality of the included studies was evaluated by the Newcastle–Ottawa scale, a widely used

FIGURE 1. Flow chart of study selection in the meta-analysis.
### TABLE 1. Characteristics of Included Studies on the Association Between C. trachomatis Infection and Cervical Cancers Risk

| Author, year          | Country  | Study Year (s) | Study Design     | Age Range, year | Specimen Source | Quality | Case group (Infection Number/Total Number) | Control Group (Infection Number/Total Number) |
|-----------------------|----------|----------------|------------------|-----------------|-----------------|---------|-------------------------------------------|-----------------------------------------------|
| Castellsagu et al (2013)¹ | Spain    | 1992–2000      | Nested case–control ¹ | 35–70           | Serum           | 9       | 40/184                                    | 30/368                                        |
| Farivar et al (2012)    | Iran     | 2007–2009      | Case–control     | 21–78           | Serum           | 6       | 0/76                                      | 3/150                                         |
| da Silva et al (2012)   | Brazil   | 2007–2009      | Case–control     | 14–68           | Serum           | 5       | 3/5                                       | 5/24                                          |
| Arnhem et al (2011)²  | Finland  | 1975–2002      | Cohort¹          | 15–60           | Serum           | 8       | 277/588                                   | 818/2846                                      |
| Naucier et al (2007)⁸  | Taiwan   | 1991–1995      | Nested case–control¹ | 30–64           | Serum           | 8       | 24/60                                     | 81/259                                        |
| Zereu et al (2007)⁹    | Brazil   | 1995–2003      | Case–control     | 15–67           | Serum           | 6       | 0/67                                      | 0/139                                         |
| Golijow et al (2005)¹⁰ | Argentina| 1998–2000      | Case–control     | 29–39           | Serum           | 8       | 645/1238                                  | 339/1100                                      |
| Schmauz et al (1993)²² | United Kingdom | 1984–1988       | Case–control     | 20–44           | Serum           | 8       | 50/71                                     | 95/153                                        |
| Reesink-Peters et al (2001)¹³ | Netherlands | 1988–1993         | Cross-sectional study | 31–42           | Serum           | 7       | 3/14                                      | 16/40                                         |
| Hare et al (1982)²³   | United Kingdom | 1984–1985       | Case–control | 40–44           | Serum           | 7       | 12/32                                     | 4/18                                          |
| Kalimo et al (1981)²⁴ | Finland  | 1984–1988      | Case–control     | 16–48           | Serum           | 7       | 2/11                                      | 1/97                                          |
| Dong et al (1998)²⁶   | China    | 1995–1996      | Cohort           | 25–72           | Cervical secretion | 7       | 9/43                                     | 53/370                                        |
| Stone et al (1995)⁴    | US       | 1982–1984      | Case–control     | 25–59           | Serum           | 7       | 373/564                                   | 384/764                                       |
| Kwasniewska et al (2009)²⁷ | Poland   | 1992–2002      | Case–control     | 44.93, 45.94²⁷ | Paraffin       | 7       | 147/570                                   | 4/50                                          |
| Hsieh et al (1999)³⁰   | Taiwan   | Unclear        | Case–control     | Unclear         | Serum           | 8       | 14/100                                    | 3/154                                         |

*Case group was patients with cervical cancer.
¹Ten European countries: Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden, and the United Kingdom.
²Prospective study.
³Four countries: Finland, Norway, Iceland, and Sweden.
⁴Seven countries: Morocco, Brazil, Peru, Colombia, Thailand, Philippines, and Spain.
⁵Median ages for all the cases.
⁶Median age for the cases and the controls.
tool for the quality assessment of observational or nonrandomized studies.”

**Statistical Analysis**

The strength of association between the *C. trachomatis* and risk of cervical cancer was estimated by OR and 95% CIs. The significance of the pooled OR was determined by Z test, with *P* < 0.05 was considered significant. Heterogeneity was quantified and evaluated by the Chi-squared-based Q-test and *I*² test, with *P* < 0.10 and *I*² > 50% indicating evidence of heterogeneity. When the effects were assumed to be homogenous, the fixed-effects model was used (Mantel–Haenszel method). If obvious heterogeneity was present, the random-effects model was used (DerSimonian–Laird method). Subgroup analysis was conducted by histological type (squamous cell cervical carcinoma and adenocarcinoma), source of specimen (serum, cervical secretion, and paraffin-embedded tissues), and study design (univariate analysis or multivariate analysis, retrospective or prospective study). Potential publication bias was assessed by funnel plots.

All analyses were conducted using Review Manager 5.3 software with forest plot, whole OR, and 95% CIs (Cochrane Review Manager Software, the Cochrane Collaboration). *P* < 0.05 was considered significant.

**RESULTS**

**Literature Search Results**

The search yielded 514 records, of them, 437 were excluded as irrelevant on the basis of title and abstract. Further assessment for more detailed information identified 77 articles, of which 57 publications were excluded because of improper study design (n = 40, including 12 articles used cervical intraepithelial neoplasia as case group or control group, 3 articles used HPV-positive women as control participants), duplicate reporting (n = 11), not in English (n = 5), and review article (n = 1).

![FIGURE 2. The association between *C. trachomatis* infection and the risk of cervical cancer in prospective studies and retrospective studies.](image-url)
Among the remaining 20 articles, 2 studies analyzed 2 distinct cohorts. One is a cross-sectional study involving 2 groups of women (group A between September 1988 and September 1993, group B between November 1995 and June 1999). The other analyzed 2 case–control studies in Spain and Colombia. The data in each of these 2 articles were extracted as 2 individual studies. Thus, 22 individual studies from 20 publications with a total of 4291 cases and 7628 controls were included in our meta-analysis. Details of the study selection process are presented in Figure 1.

Characteristics of Included Studies

The 22 studies on *C. trachomatis* and the risk of cervical cancers were published between 1981 and 2015. Most of these studies were performed in 1 country, except 3, which involved multiple countries. Among those 22 studies, 19 were retrospective, while the other 3 were prospective. All studies included in this meta-analysis were prevalence studies. Most of them were case–control and cross-sectional studies, assessing *C. trachomatis* infection and cervical cancer prevalence at a given point of time. Only 3 studies had a longitudinal design and assessed prevalence data with a median followed up period of 9 years, 9.5 years, and 9 years, respectively.

All of the 22 studies were performed with univariate analysis, in which 11 of them were adopted with multivariate analysis adjusted by age, use of oral contraceptives, history of smoking, and other factors. All of the multivariate analyses were adjusted for HPV and age.

In addition, eleven studies reported the association between *C. trachomatis* and squamous cell carcinomas (SCC) and 5 studies involved adenocarcinoma in cervix. The quality score of the included studies ranged from 6 to 9 stars according to the 9-star Newcastle–Ottawa scale. Their main characteristics are presented in the Table 1.

Main Analysis

The overall prevalence of *C. trachomatis* infection in women with cervical cancer and controls in this study was 41.95% and 26.27%, respectively. Meta-analysis of total eligible studies showed that there was a significant link between *C. trachomatis* and the risk of cervical cancer in prospective studies (OR = 2.21, 95% CI: 1.88–2.61, *P* < 0.001) (Figure 2), as well as in retrospective studies (OR = 2.19, 95% CI: 1.74–2.74, *P* < 0.001) (Figure 2).

*C. trachomatis* was identified as an independent predictor of cervical cancer (OR = 1.76, 95% CI: 1.03–3.01, *P* = 0.04) in 11 studies using multivariate logistic regression analysis adjusted for HPV and age (Figure 3), in which 6 studies also were adjusted for oral contraceptive, 6 studies were adjusted for sexual status such as number of sexual partners,
age at 1st intercourse, age at 1st birth, and number of full-term pregnancies, and 4 studies were adjusted for history of smoking. Moreover, 6 studies evaluated the coinfection of HPV and *C. trachomatis* and suggested that coinfection of HPV and *C. trachomatis* was related to a higher risk of uterine cervix cancer (OR = 4.03, 95% CI: 3.15–5.16, *P* < 0.001) (Figure 3).

**Subgroup-Analysis**

A subgroup analysis based on histological type indicated a higher risk of both SCC (OR = 2.21, 95% CI: 2.00–2.45, *P* < 0.001) and adenocarcinoma (OR = 1.61, 95% CI: 1.21–2.15, *P* = 0.001) in the cervix associated with *C. trachomatis* infection (Figure 4).

Analysis according to specimen source indicated that an increased risk of cervical cancer was related to the infection of *C. trachomatis* detected in various specimen including serum (OR = 2.20, 95% CI: 2.01–2.42, *P* < 0.001), paraffin embedded cervical tissue (OR = 2.88, 95% CI: 1.21–6.83, *P* = 0.02), and cervical secretion (OR = 2.71, 95% CI: 1.41–5.20, *P* = 0.003) (Figure 5). However, 2 studies showed that none of cervical cancer cases was positive for *C. trachomatis* with polymerase chain reaction assay using DNA extracted from paraffin-embedded tissue specimens of patients with cervical cancer.5,7

**Publication Bias**

Publication bias was investigated by the funnel plots. As shown in Figure 6, there was low possibility of bias on visualization of funnel plots in this meta-analysis.

**DISCUSSION**

*C. trachomatis* is a gram-negative, obligate intracellular bacterium that infects human epithelial cells of the genital tract as well as ocular tissue. Genital *C. trachomatis* infection may result in pelvic inflammatory disease, ectopic pregnancy, and infertility in some cases, and is also implicated to increase the risk of cervical neoplasia.15,16,28,29 Whether *C. trachomatis* infection truly stimulates cervical carcinoma is one of the most pressing questions in this field of research.

In this meta-analysis of 19 retrospective studies and 3 prospective studies (with a total of 4291 cases and 7628 controls), we confirmed that *C. trachomatis* infection was significantly associated with increased risk of cervical cancer. The finding that the presence of antichlamydial heat shock protein 60–1 antibodies was associated with an increased cervical cancer risk also supports the correlation between persistent *C. trachomatis* infection and cervical neoplasia.30 A plausible mechanism for chlamydial infection to increase cervical cancer risk is the infection-associated inflammatory response, thus leading to reactive oxidative metabolite production, increased expression of cytokines, chemokines, and growth and angiogenic factors, decreased cell-mediated immunity, and the generation of free radicals, all of which can cause damages to DNA and impair DNA repair function resulting in genetic instability.31,32

*C. trachomatis* infection triggered the production of supernumerary centrosomes and chromosome segregation defects, facilitated multipolar mitosis, actively promoted chromosome instability, caused multinucleation, and thereby led to transformation and tumor development.33–36 Additionally, *C. trachomatis* disrupted N-cadherin-dependent cell-cell junctions and caused the breakdown

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**FIGURE 4.** The association between *C. trachomatis* infection and the risk of cervical cancer subgrouped by histological type.
of the N-cadherin/β-catenin complex in primary cultures of human cervical epithelial cells and in HeLa cells. More recently, Discacciati et al.38 found Matrix metalloproteinases-9/Reversion-inducing Cysteine-rich protein with Kazal motifs (RECK) imbalance during cervical inflammation induced by C. trachomatis might play a role in cervical carcinogenesis. In the same line of evidence it was shown that infection of mice with C. trachomatis resulted in significantly increased cell proliferation, within the cervix, and in evidence of cervical dysplasia.39

Infection with HPV is established as a major cause of cervical cancer. A large body of evidence suggests that C. trachomatis infection may increase the risk of HPV acquisition as well as HPV persistence.40–42 Two recent studies have found that the history of C. trachomatis infection appears to increase the probability of persistent HPV infection.43,44 A recent prospective, population-based cohort study with up to 19 years of follow-up showed that repeated C. trachomatis infection increased the risk of cervical intraepithelial neoplasia 3+ among

FIGURE 5. The association between C. trachomatis infection and the risk of cervical cancer subgrouped by specimen source.

FIGURE 6. Funnel plot for the assessment of the publication bias Checklist S1 PRISMA 2009 checklist. (DOC).
women with prevalent as well as persistent high-risk HPV infection.42 Similarly, our research indicated that coinfection of HPV and C. trachomatis was related to a higher risk of uterine cervical cancer, further strengthening this relationship. It may be due to 2 mechanisms. First, HPV infection in the basal keratinocytes of the mucosal epithelium requires the presence of microabrasions or altered epithelium. Chlamydial infection could possibly lead to epithelial disruption, thus, facilitating the entry of the virus.45,46 Second, chlamydial infection might also disturb the immune response necessary to clear the virus.11,32

However, the infections both of HPV and C. trachomatis are sexually transmitted. They have similar behavioral risk factors, such as younger age and higher numbers of sexual partners. As a result, the 2 infections could occur concurrently, rather than C. trachomatis infection directly affecting HPV acquisition. Epidemiologic evidence have demonstrated a higher risk of cervical cancer for women with antibodies against C. trachomatis, both in analyses that have adjusted for HPV infection and in stratified analyses where only HPV DNA-positive cases and controls have been included.17,41 In this meta-analysis, multivariate logistic regression analysis was performed in 11 studies and the meta-analysis confirmed a higher risk of developing cervical cancer in patients with C. trachomatis infection, even after adjustment for HPV infection. Consequently, we believe that the C. trachomatis infection is not likely to be a simple comorbidity with HPV infection, but should be an independent predictor for cervical cancer risk.

Results of several previous studies suggested the role of C. trachomatis as a carcinogenic cofactor may be restricted to cervical SCC. Several studies found C. trachomatis infection was a risk factor for invasive cervical SCC, but not for adenocarcinoma.17,48 Similarly, 2 prospective studies showed the association between C. pneumoniae infection and invasive SCC, but not with adenocarcinoma of the lung.49,50 In this study, it is an interesting result to find that C. trachomatis infection is associated with a higher risk in both SCC and adenocarcinoma of the cervix. Given endocervix (together with the squamocolumnar junction) is the major site of C. trachomatis infection in the lower genital tract, we hypothesize that C. trachomatis infection may play a role in the initiation of malignant transformation of the glandular endocervical epithelium via the above-outlined mechanisms related to HPV coinfection. In addition, C. trachomatis may play a role in the progression of adenocarcinoma by evoking the inflammatory response that damages the mucosal barrier including the basement membrane.51 A further study is recommended in the domain of the effect of C. trachomatis in adenocarcinoma carcinogenesis of cervix.

Elevated risks of cervical cancer were indicated in C. trachomatis infection in various specimen sources including cervical secretion, serum, and paraffin embedded cervical tissue, especially in serum with no obvious issue of heterogeneity. Therefore, C. trachomatis detection in serum may be a possible method to predict the risk of cervical cancer in clinic. However, 2 studies showed none of the cases was positive for C. trachomatis with polymerase chain reaction assay using DNA extracted from paraffin-embedded cervical tissue specimens of patients with cervical cancer. On the basis of the outcomes in this study, we believe that the polymerase chain reaction assay using paraffin-embedded cervical tissue specimens is not a reliable way to evaluate the infection of C. trachomatis.

Our study has some limitations. Firstly, most of our quantitative assessment studies were based on case–control studies where data on prevalence of C. trachomatis and cervical cancers were acquired simultaneously, rather than longitudinally. None of the studies have taken into account the association between the duration of C. trachomatis infection and the risk of cervical cancer. Another limitation is that a meta-analysis is impossible to tackle the problems of confounding factors that could be inherent in the included studies. Inadequate control for confounders may bias the results in overestimation or underestimation of risk estimates. Moreover, heterogeneity may be introduced because of methodological differences among studies, including different specimen sources. Although there was low possibility of bias on visualization of funnel plots in this meta-analysis, the retrieved literature might potentially not be comprehensive enough. Studies with a statistically significant effect are more likely to be published and to be cited by other authors, while the results showed no association between C. trachomatis and cervical cancer may be unpublished.

The underlying interaction between C. trachomatis and cervical cancer risk needs to be confirmed in longitudinal studies. Thus, our study calls for further investigation in more prospective studies to provide more definitive evidence concerning the role of this pathogen as a promoter of HPV-mediated cervical carcinogenesis.

In summary, this meta-analysis strengthens the evidence that infection with C. trachomatis could be one of the risk factor of cervical cancer. Individuals infected with C. trachomatis have a heightened risk of developing cervical cancer. Therefore, it is necessary to expand C. trachomatis infection screening and treat women with C. trachomatis infection timely, particularly among women at a higher risk of HPV infections. This approach will not only protect against pelvic inflammatory disease and infertility, but potentially also prevent cervical cancer and reduce the incidence of cervical cancer.

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