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Infections of *Chlamydia trachomatis* and *Mycoplasma hominis* as Risk Factors for Abnormal Cervical Cells

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**Abstract**

**Background:** Cervical cancer is the fourth most common cancer among women across the world. Recent studies have shown that cervical cancer is not only caused by persistent infection of human papillomavirus (HPV), but sexually transmitted infections (STIs) also play a role in the pathogenesis of abnormal cervical cells. STIs frequently occur with no specific symptoms, such as the infections caused by *Chlamydia trachomatis* and *Mycoplasma hominis*. Asymptomatic STIs could lead to persistent infection. Persistent infections caused by STIs have been hypothesised to increase the access of HPV into the deeper cervical tissue and cause cervical cell abnormalities. Therefore, we conducted this study to assess the association between *C. trachomatis* and *M. hominis* bacterial infections and abnormal cervical cells. **Methods:** A cross-sectional study was performed on 58 outpatients at the Department of Obstetrics and Gynecology, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia. Abnormal cervical cells were detected by a liquid-based cytology (LBC) Pap smear, and bacterial identification was done by conducting conventional duplex polymerase chain reaction (PCR). **Results:** 58 patients, 14 (24.1%) showed abnormal cervical cells, whereas 44 (75.9%) patients showed normal cervical cells. The conventional duplex PCR demonstrated a positive result for *C. trachomatis* and *M. hominis* bacterial infections in only 1 (7.1%) and 2 (14.3%) patients with abnormal cervical cells, respectively. The statistical analysis revealed no significant association between the bacterial infections and the abnormal cervical cytology in the patients (p > 0.05). **Conclusions:** Infections caused by *C. trachomatis* and/or *M. hominis* were not associated with abnormal cervical cells.

**Keywords:** cervical cancer, *Chlamydia trachomatis*, human papillomavirus (HPV), *Mycoplasma hominis*, sexually transmitted infections

**Introduction**

Cervical cancer is the fourth most common malignancy among women across the world, and it is the second leading cause of female cancer in Indonesia.¹ Reports on human papillomavirus (HPV) and related diseases from Indonesia have shown that the incidence of cervical cancer is 20,928 cases/year with 9,498 deaths/year.¹ These data indicate that the morbidity and the mortality of cervical cancer are high, wherein the mortality rate is about 50% of the morbidity rate each year.

Persistent infections caused by HPV have been reported to be the primary aetiology of pre-cancerous lesions and cervical cancer.²,³ HPV was detected in 90% of cervical cancer cases, with the majority being HPV-16 and -18 types.²,³ Cervical cancer, which is initiated by pre-cancerous lesions, can be detected using a liquid-based cytology (LBC) Pap smear.⁴ These pre-cancerous lesions are caused by persistent HPV infections, and they are also involved in other risk factors, such as other sexually transmitted infections (STIs).⁵,⁶ STIs have been hypothesised as risk factors for pre-cancerous cervical lesions, because these infections could cause persistent infections with no specific symptoms. These persistent infections can increase the access of HPV into the deeper cervical tissue.⁵,⁶
Among STIs, several studies have reported that infections caused by *C. trachomatis* and *M. hominis* play an important role in the persistence of HPV infection, leading to cervical cancer.5–7 *C. trachomatis* infection could inhibit the apoptosis of infected cells,5,8 whereas *M. hominis* infection could suppress the cell-mediated immunity.9,10 These mechanisms render the infected cervical cells to become more susceptible to HPV infection and lead to HPV persistence.9 A study in Korea reported that *C. trachomatis* infections were significantly higher in abnormal cervical cells than in normal cells.6 Another study from Argentina demonstrated that *C. trachomatis* infections were higher in HPV-positive (34.2%) than in HPV-negative subjects (19%).5 *M. hominis* was detected in 5.33% of abnormal cervical cells.9 Yet another study from Nigeria showed a significant association between *M. hominis* and persistent HPV.10 To our knowledge, there is no report about the roles of *C. trachomatis* and *M. hominis* infections as risk factors for abnormal cervical cells in patients from Indonesia. Therefore, we conducted this cross-sectional study to determine the association between *C. trachomatis* and *M. hominis* infections and abnormal cervical cells.

Methods

Study Population. A total of 58 female outpatients attending the Colposcopy Outpatient Clinic, Department of Obstetrics and Gynecology, Dr. Cipto Mangunkusumo Hospital (RSCM), Jakarta, Indonesia, during June to October 2016, were included in this cross-sectional study. The participants were aged 20–67 years and were sexually active. Patients who were pregnant and received antibiotics during the past 4 weeks were excluded. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia, with no: 98/UN2.F1/ETIK/2016, and all subjects provided the written informed consent for participation in the study.

Specimens. Samples of cervical swabs collected by specialist doctors were immediately inserted into LBC vials (LiquiPrep, LGM International Inc, USA). The samples were aliquoted into two tubes, each 5 mL, for LBC Pap smear and polymerase chain reaction (PCR). Transportation and storage of the samples were maintained at 4 °C, and the samples were processed within not more than 2 days from the day of collection.

LBC Pap Smear. LBC Pap smears were performed according to the manufacturer’s protocol (LiquiPrep, LGM International Inc, USA) under the supervision of a pathologist. Normal and abnormal cells were classified according to the Bethesda classification system as follows: negative for intra-epithelial lesions or malignancy (NILM), atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells-cannot exclude HSIL (ASCH), low-grade squamous intra-epithelial lesion (LSIL) and high-grade squamous intra-epithelial lesion (HSIL).11 The ASCUS, ASCH, LSIL and HSIL were classified as results depicting an abnormal cervical cytology.

DNA Extraction. One millilitre of samples was centrifuged at 13,000 rpm for 30 min. The pellet was suspended in 200 µL of phosphate-buffered saline (PBS) and extracted in accordance with the manufacturer’s protocol (High Pure PCR Template Preparation Kit, Roche, Germany) using 50 µL of the final elute. Sample collection and DNA extraction were validated by PCR conducted using standard β-globin primers (Figure 1).

![Figure 1. Human β-Globin (266 bp) Detection Using Conventional PCR. M: Marker; P: Positive Control; N: Negative Control; S1–S7: Samples; bp: Base Pair](image-url)
PCR for Detecting \textit{C. trachomatis} and \textit{M. Hominis}. \textit{C. trachomatis} and \textit{M. hominis} were detected by conducting conventional duplex PCR using the following primers reported by Dhawan et al.\textsuperscript{12} and Pascual et al.\textsuperscript{13}: \textit{C. trachomatis} with a product size of 71 bp (Ct forward 5’-CATGAAAACTCGTTCCGAAATAGAA-3’ and Ct reverse 5’-TCAGAGCTTATACCTAACAACCGATA-3’) and \textit{M. hominis} with a product size of 101 bp (Mh forward 5’-TTTGGTCAAGTCCTGCAACGA-3’ and Mh reverse 5’-CCCCACCTTCTCCCAGTTA-3’). The PCR was performed using a total volume of 20 µL containing 10× PCR buffer, 1.2 mM MgCl\textsubscript{2}, 0.2 mM dNTP mix, 5× Q solution, 0.05 µM primer \textit{M. hominis}, 0.45 µM primer Ct\textsubscript{F}, 0.15 µM primer Ct\textsubscript{R}, 1.25 U of the enzyme Taq polymerase (HotStarTaq DNA Polymerase, Qiagen) and 4 µL of DNA sample. Thermal cycles of PCR were performed using a Biosystems GeneAmp PCR System 2700 (Applied Biosystems, USA) at 95 °C for 15 min, with 40 amplification cycles at 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s. The final elongation step was performed at 72 °C for 7 min.

The PCR products were analysed on 9% polyacrylamide gel electrophoresis (PAGE), and the DNA bands were documented by UV transillumination (GelDoc\textsuperscript{TM} XR, Biorad).

\textbf{Statistical Analysis.} The statistical analysis was performed by Fisher’s exact test using the IBM SPSS (version 20) software package (IBM Corp., USA). Data were reported as numbers (percentages) with 95% confidence intervals. The results were considered to be significant when \( p \leq 0.05 \).

\textbf{Results}  
In total, 58 patients who participated in this study, 44 (75.9\%) and 14 (24.1\%) patients were diagnosed with normal (NILM) and abnormal cervical cytology results, respectively. Of the 14 patients with an abnormal cervical cytology, 6 (12.5\%) had ASCUS, 4 (8.3\%) had ASCH, 1 (2.1\%) had LSIL and 3 (6.3\%) patients had HSIL.

\begin{figure}[h]  
\centering  
\includegraphics[width=\textwidth]{figure2.png}  
\caption{\textit{C. trachomatis} (71 bp) and \textit{M. hominis} (10 bp) Detection Using Conventional Duplex PCR. M: Marker; P: Positive Control; N: Negative Control; S1–S7: Samples; bp: base pair}  
\end{figure}

\begin{table}[h]  
\centering  
\begin{tabular}{|l|c|c|c|}
\hline  
\textbf{Results} & \textbf{Negative cytology (NILM) n (%)} & \textbf{Abnormal cytology n (%)} & \textbf{p*} \\
\hline  
\textit{C. trachomatis} & & & \\
Positive & 0 (0) & 1 (7.1) & 0.241 \\
Negative & 44 (100) & 13 (92.9) & \\
\hline  
\textit{M. hominis} & & & \\
Positive & 3 (6.8) & 2 (14.3) & 0.348 \\
Negative & 41 (93.2) & 12 (85.7) & \\
\hline  
\end{tabular}  
\caption{Cytopathological Results of The Screening of Indonesian Women with and Without \textit{C. trachomatis} and \textit{M. hominis} Infections}  
\end{table}

NILM: negative for intra-epithelial lesion or malignancy. Abnormal cervical cytology includes ASCUS, ASCH, LSIL and HSIL. ASCUS: atypical squamous cells of undetermined significance. ASCH: atypical squamous cells-cannot exclude HSIL. LSIL: low-grade squamous intra-epithelial lesion. HSIL: high-grade squamous intra-epithelial lesion. n: number of patients. The comparison between cases with positive and negative test results according to Fisher’s exact test.
The conventional duplex PCR results (Figure 2) revealed the presence of *C. trachomatis* in 1 of the 14 patients with an abnormal cervical cytology, whereas *C. trachomatis* infection was not detected in the 44 patients with NILM results (Table 1). *M. hominis* was detected in 2 of the 14 patients with an abnormal cervical cytology and in 3 of the 44 patients with NILM results. As shown in Table 1, no significant association was found between *C. trachomatis* and/or *M. hominis* infection and abnormal cervical cytology, with the p values being 0.241 and 0.348, respectively.

**Discussion**

HPV, especially the high-risk genotypes, is known as an aetiological agent causing cervical cancer disease. However, the changes from normal to cancerous cervical cells are influenced by several risk factors.\(^5,6\) One of the risk factors is sexually transmitted bacterial infections, including the infections causing by *C. trachomatis* and *M. hominis*.\(^5,6\) The specific role of bacterial infections in regulating the changes in cervical cells of HPV-infected women has not yet been delineated. A possible explanation is that the bacterial infections may increase the susceptibility of women to HPV infection through changes in cervical cells leading to a higher susceptibility to HPV infection.\(^5,16\) Several studies have demonstrated the association between bacterial infections and the development of abnormal cervical cells.\(^5,6,14,15\)

*C. trachomatis* has been reported to be the topmost aetiological agent causing STIs across the world, with approximately 89 million cases/year.\(^7\) This bacterial infection has also been reported to play an important role in the development of abnormal cervical cells. These infections might lead to chronic and persistent infections and inflammation, leading to the inhibition of cell apoptosis and metaplasia in cervical squamous cells, thereby rendering the cells susceptible to HPV infection.\(^5,16\) Moreover, the persistent infection might lead the persistence of HPV, which is an indicator for the development of cervical cancer.\(^17\)

In Indonesia, the prevalence of *C. trachomatis* infection has been reported to be about 7.9%.\(^18\) However, there is no information regarding the association between *C. trachomatis* infection and abnormal cervical cells. In the present study, we found no significant association between *C. trachomatis* and abnormal cervical cytology, with the p value being 0.241. This result was consistent with that reported by de Castro-Sobrinho *et al.*\(^19\) with the p value being 0.2 in their study. In contrast, another study reported that abnormal cervical cells showed a higher percentage of positive *C. trachomatis*.\(^6\) This difference in results might be caused due to several factors, in particular, the local customs, the behaviour and the risk exposure of particular women.\(^20\)

Besides *C. trachomatis*, we also investigated the role of *M. hominis* as a risk factor for the development of abnormal cervical cells. The bacterium can be found as a commensal agent in healthy women, but it can be a harmful agent in sexually active women.\(^9,21\) Several studies have demonstrated the association between *M. hominis* infection and cervical problems.\(^21-23\) Indeed, a study reported that *M. hominis* infection could suppress the cell-mediated immunity, an important protective mechanism against HPV infection.\(^9,20\) However, in this study, we found no such association between *M. hominis* infection and abnormal cervical cells (p = 0.348). Although there was no statistically significant association, the percentage of *M. hominis* in patients with an abnormal cervical cytology was higher (14.3%) than that in patients with a normal cervical cytology (7%). Similarly, other studies conducted by Farag *et al.*\(^9\), Choi *et al.*\(^6\) and Kim *et al.*\(^24\) also showed no significant association between *M. hominis* and abnormal cervical cytology.

A limitation of this study is that we did not analyse the association between *C. trachomatis* and/or *M. hominis* and infections caused by HPV, an aetiological virus causing cervical cancer disease. In addition to these bacterial infections, it is necessary to include STIs caused by other bacteria such as *Ureaplasma urealyticum* and *U. parvum* in future studies.

Moreover, further research using a larger population must be conducted. The status of other STI-causing agents and HPV infection may be used as a research variable. The findings of this study may be used as information to improve the quality of treatment of patients with pre-cancerous cervical lesions, by including the microbiological examination of women with an abnormal cervical cytology.

**Conclusions**

This study demonstrated that *C. trachomatis* and/or *M. hominis* infections have no significant association with an abnormal cervical cytology. However, it is known that HPV infections alone do not influence the changes from normal to abnormal cervical cells. There are several risk factors influencing the cellular changes, one of them being sexually transmitted bacterial infections. Therefore, it is necessary to address other sexually transmitted bacterial infections besides *C. trachomatis* and *M. hominis* in future studies.

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Conflict of Interest Statement
We declare no conflict of interest.

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