Increased Risk of Endometriosis in Patients With Lower Genital Tract Infection

A Nationwide Cohort Study

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Abstract: Endometriosis results from the ectopic invasion of endometrial glands and stroma in the peritoneal cavity. The exact etiology of endometriosis is still unknown. It has, however, been shown that there are higher numbers of *Escherichia coli* in menstrual blood, and higher endotoxin levels in menstrual fluid, as well as, in the peritoneal fluid of patients with endometriosis. In this study, we aimed to determine whether lower genital tract infections could increase the risk of endometriosis.

We used the Taiwan National Health Insurance database to conduct a population-based cohort study. We included patients diagnosed with inflammatory diseases of the cervix, vagina, and vulva, and a control group comprising patients matched by age, sex, and comorbidities but without inflammatory diseases of the cervix, vagina, or vulva.

A total of 79,512 patients were included in the inflammatory disease group and an equal number of control individuals were selected. The incidence of endometriosis (hazard ratio, 2.01; 95% confidence interval, 1.91–2.12; P < 0.001) was higher among patients than controls. Cox proportional hazards models showed that irrespective of comorbidities, lower genital tract infection was an independent risk factor for endometriosis.

Patients with lower genital tract infections exhibit a substantially higher risk for developing endometriosis.

INTRODUCTION

Endometriosis is characterized by the existence of endometrial glands and stroma outside the uterine cavity. Patients with endometriosis have common symptoms, such as dysmenorrhea, pelvic pain, and reduced fertility, and the condition presently affects up to 10% of all menstruating women.1–4

The exact etiology of endometriosis remains uncertain; however, the most acceptable hypothesis is retrograde menstruation of viable endometrial tissues infiltrating the peritoneal cavity, which subsequently leads to implantation and invasion establishing pelvic endometriosis.5–9 The ectopic endometrial tissues promote chronic inflammatory responses.10 However, retrograde menstruation may not explain all cases of endometriosis. The differences in biochemical and pathological properties between endometrial tissues found in the ovaries, rectovaginal septum, or peritoneum indicate that endometriosis may result from distinct conditions.

Recently, inflammation has been recognized as an important factor in the pathogenesis of endometriosis.9 The ectopic endometriotic tissues secrete cytokines and chemokines to promote inflammation and to attract macrophages into the peritoneal cavity, which further stimulates the inflammatory response.10 There are increased numbers of activated macrophages found in the peritoneal cavity of endometriosis patients.11 The expression levels of monocyte chemotactic protein 1 (MCP-1), interleukin (IL)-8, IL-6, and tumor necrosis factor (TNF)-α have also been found to be increased in the peritoneal cavities of patients with endometriosis.12,13

Inflammation can also be triggered by bacterial endotoxins, which promote the secretion of inflammatory cytokines and chemokines.14 Bailey et al.15 found that the levels of intestinal microflora were altered in rhesus monkeys with endometriosis, exhibiting lower concentrations of lactobacilli but higher concentrations of gram-negative bacteria. In patients with endometriosis, there are higher numbers of *Escherichia coli* in menstrual blood, and higher endotoxin levels in menstrual fluid, as well as, in peritoneal fluid.16 Endotoxins could activate macrophages via the toll-like receptor 4 (TLR4) protein to...
promote the secretion of inflammatory cytokines. The lower genital tract in humans is invariably exposed to various bacteria and the imbalance of microflora may cause an infection, which could then migrate to the upper genital tract. To reveal the important influence of genital tract infections, we conducted a nationwide prospective cohort study to determine whether genital tract infections increase the risk of endometriosis.

**METHODS**

**Data Sources**

The National Health Insurance Administration promoted the Taiwan National Health Insurance program, which provided coverage for over 23 million residents in Taiwan, on March 1, 1995. The present study used claims data from the National Health Insurance Research Database (NHIRD), established by the National Health Research Institutes (NHRI) with authorization from the Bureau of National Health Insurance, Department of Health. The Longitudinal Health Insurance Database 2000 (LHID 2000) comprises a random sample of claims data from one million subjects in the NHIRD, with data available from 1996 through 2011. There were no significant differences in the distributions of sex or age between the original data and sampled data. Every individual in Taiwan had a unique personal identification number (PIN) code. All the databases can be interlinked through individual PIN codes. Ambulatory care claims contain the individual date(s) of visit, and inpatient claims contain the individual date(s) of visit, and International Classification of Disease, Ninth Revision, Clinical Modification (ICD-9-CM) code(s). Inpatient claims contain ICD-9-CM codes from primary diagnosis to 4 secondary diagnoses.

The NHIRD is authorized for research and medical purposes in Taiwan, and the present study was approved by the institutional review board of the China Medical University Hospital (CMUH104-REC2-115). The identification number of each patient was encrypted for privacy protection, and the informed consent process was then waived.

**Study Design and Population**

A retrospective cohort study design was used and the dataset consisted of 2 cohorts (individuals with inflammatory diseases of the cervix, vagina, or vulva, and individuals without disease). Patients with inflammatory diseases of the cervix, vagina, or vulva were identified from the database based on specific ICD-9-CM codes recorded between 2000 and 2004 and during follow-up until December 31, 2011, or if there was a new diagnosis of endometriosis during the follow-up period. For inclusion in the inflammatory disease cohort, patients were required to have at least 3 ambulatory claims, or at least 1 inpatient claim, between 2000 and 2004, with an ICD-9-CM code from 616.0X to 616.9X. We excluded patients younger than 20 years or older than 55 years, and we also excluded patients with a diagnosis of endometriosis before 2000. The index date for the inflammatory disease cohort was considered the date of the first outpatient visit or inpatient visit during the years 2000 to 2004. We retrieved 79,512 patients from the database with a diagnosis of inflammatory disease of the cervix, vagina or vulva, who were subsequently included in the inflammatory disease cohort.

An additional 79,512 age- and sex-matched individuals, without inflammatory diseases of the cervix, vagina, or vulva, were also identified from the same database to be included as study controls. For comparison purposes, and as there was no index date that could be assigned for individuals in this group, we randomly assigned a corresponding “pseudo-diagnosis date” to each control individual such that individuals in both groups were enrolled at a similar time. The exclusion criteria for the controls were the same as those stated above for patients in the inflammatory disease cohort (Figure 1).

**Study Outcome**

The primary outcome was a new diagnosis of endometriosis (ICD-9-CM: 617), which was defined as the first outpatient or inpatient visit after the index date during the follow-up. Gynecologists made the diagnoses of endometriosis by gynecological ultrasonography or laparoscopy. The time of follow-up began with the index date and ended when there was a new diagnosis of endometriosis or on December 31, 2011.

**Baseline Characteristics and Comorbidities**

Study participants were divided into 4 age categories: 20 to 29 years, 30 to 39 years, 40 to 49 years, and 50 to 55 years. Comorbidities included infertility (ICD-9-CM: 628), leiomyoma of the uterus (ICD-9-CM: 218), autoimmune diseases, allergic diseases, and cancer. Autoimmune diseases included systemic lupus erythematosus (ICD-9-CM: 710.0), rheumatoid arthritis (ICD-9-CM: 714.0), and multiple sclerosis (ICD-9-CM: 340). Allergic diseases included asthma (ICD-9-CM: 477), and allergic rhinitis (ICD-9-CM: 477). Cancer included breast cancer (ICD-9-CM: 174), cervical cancer (ICD-9-CM: 180), ovarian cancer (ICD-9-CM: 183.0), and melanoma (ICD-9-CM: 172.9). Only diagnoses made before, or in concurrence with, the index date were regarded as underlying comorbidities.

**Statistical Analysis**

Comparisons between groups were performed using Pearson $\chi^2$ test for categorical variables, as appropriate. We estimated the cumulative risk of endometriosis for both cohorts using the Kaplan–Meier method, and the significance of the cumulative risk curves was assessed by the log-rank test. The Cox proportional hazards model was used to calculate the hazard ratio (HR) and 95% confidence interval (CI) of endometriosis in patients with inflammatory diseases of the cervix, vagina, or vulva in comparison to those of controls. All analyses were carried out with SAS statistical software (version 9.4 for Windows; SAS Institute Inc, Cary, NC). Statistical significance was determined as $P < 0.05$.

**RESULTS**

A total of 79,512 patients were included in the inflammatory disease group, and an equivalent number of control individuals were included in the study. Table 1 summarizes the baseline characteristics and comorbidity status of the study groups. The mean age of inflammatory disease cohort was 34.70 ± 9.17 years and that of the controls was 34.61 ± 9.62 years. The mean follow-up period was 9.29 years (median, 9.62 years) and 8.97 years (median, 9.72 years) for patients with inflammatory diseases and control patients, respectively. The prevalence of infertility, leiomyoma of the uterus, and allergic diseases was significantly higher in those with inflammatory diseases of the cervix, vagina, or vulva.

Table 2 displays the results of univariate and multivariate Cox proportional hazards models. The adjusted HRs of endometriosis was significantly higher for patients with inflammatory diseases of the cervix, vagina, and vulva (HR = 2.01, 95% CI = 1.91–2.12). Patients with comorbid infertility (HR = 1.44, 95% CI = 1.22–1.71), leiomyoma of the uterus (HR = 2.65, 95% CI = 2.32–3.00), and allergic rhinitis (HR = 1.42, 95% CI = 1.31–1.53) were also at an increased risk of endometriosis.
95% CI = 2.37–2.97), and allergic diseases (HR = 1.22, 95% CI = 1.12–1.32) were also at higher risk of endometriosis in comparison to controls.

A total of 4305 and 2009 individuals were newly diagnosed with endometriosis during the follow-up period in the disease cohort and control cohort, respectively. The incidence rate in the disease cohort was 5.83 per 1000 person-years and 2.82 per 1000 person-years in the control cohort. We further explored the association between inflammatory diseases of the cervix, vagina, and vulva and endometriosis by stratifying age and comorbidities (Table 3). The adjusted HRs of endometriosis among patients with inflammatory diseases of the cervix, vagina, and vulva, indicating higher risk, were observed in individuals aged 20 to 29 years, 30 to 39 years, 40 to 49, and 50 to 55 years, as well as, in those with and without leiomyoma of the uterus, those with and without allergic diseases, and those without infertility, autoimmune disease, or cancer.

The joint effect(s) of inflammatory diseases of the cervix, vagina, and vulva and comorbid infertility, leiomyoma of the uterus, autoimmune diseases, allergic diseases, and cancer are shown in Table 4. Inflammatory diseases of the cervix, vagina, and vulva in patients with infertility (HR = 2.88, 95% CI = 2.36–3.50), leiomyoma of the uterus (HR = 4.90, 95% CI = 4.28–5.60), autoimmune diseases (HR = 2.15, 95% CI = 1.45–3.19), and allergic diseases (HR = 2.35, 95% CI = 2.13–2.60) exhibit higher risk for endometriosis.

Kaplan-Meier analysis was used to compare the incidence of endometriosis between the inflammatory disease cohort and
### TABLE 1. Demographic Characteristics and Comorbidities in Patients With and Without Inflammatory Diseases of the Cervix, Vagina, and Vulva

| Variables                          | No (N = 79512) | Yes (N = 79512) | P* |
|------------------------------------|----------------|----------------|----|
| **Inflammatory Diseases of Cervix, Vagina, and Vulva** |                |                |    |
| Age, y (means ± SD)1)              |                |                |    |
| 20–29                              | 29083 (36.58)  | 29083 (36.58)  | 0.99 |
| 30–39                              | 25985 (32.68)  | 25985 (32.68)  |    |
| 40–49                              | 19716 (24.8)   | 19716 (24.8)   |    |
| 50–55                              | 4728 (5.95)    | 4728 (5.95)    |    |
| Comorbidity                        |                |                |    |
| Infertility                        | 470 (0.59)     | 1336 (1.68)    | <0.0001 |
| Leiomyoma of uterus                | 1141 (1.44)    | 2311 (2.91)    | <0.0001 |
| Autoimmune disease                 | 399 (0.50)     | 447 (0.56)     | 0.098 |
| Allergic disease                   | 5720 (7.19)    | 7909 (9.95)    | <0.0001 |
| Cancer                             | 255 (0.32)     | 232 (0.29)     | 0.02966 |
| Outcome                            |                |                |    |
| Endometriosis                      | 2009 (2.53)    | 4305 (5.41)    | <0.0001 |
| Follow-up period (mean, median)    | 8.97 (9.72)    | 9.29 (9.88)    |    |

* χ² test.  
1 Two-sample t test.

### TABLE 2. Cox Model Measured HR and 95% CI for Endometriosis Risk in Patients With and Without Inflammatory Diseases of Cervix, Vagina, and Vulva

| Characteristics | Crude          | Adjusted         | Crude          | Adjusted         |
|-----------------|----------------|------------------|----------------|------------------|
|                 | HR (95% CI)    | P                | HR (95% CI)    | P                |
| Inflammatory diseases of cervix, vagina, and vulva |                |                  |                |                  |
| No              | 1.00 Reference |                  | 1.00 Reference |                  |
| Yes             | 2.07 (1.97–2.19) <0.0001 |                  | 2.01 (1.91–2.12) <0.0001 |
| Age, y (means ± SD) |                |                  |                |                  |
| 20–29           | 1.00 Reference |                  | 1.00 Reference |                  |
| 30–39           | 1.60 (1.51–1.69) <0.0001 |                  | 1.57 (1.48–1.66) <0.0001 |
| 40–49           | 1.07 (1–1.14)  0.0677 |                  | 1.00 (0.94–1.08) 0.9402 |
| 50–55           | 0.19 (0.15–0.25) <0.0001 |                  | 0.18 (0.14–0.23) <0.0001 |
| Comorbidity     |                |                  |                |                  |
| Infertility     | 1.00 Reference |                  | 1.00 Reference |                  |
| Yes             | 1.98 (1.68–2.35) <0.0001 |                  | 1.44 (1.22–1.71) <0.0001 |
| Leiomyoma of uterus |                |                  |                |                  |
| No              | 1.00 Reference |                  | 1.00 Reference |                  |
| Yes             | 2.70 (2.42–3.01) <0.0001 |                  | 2.65 (2.37–2.97) <0.0001 |
| Autoimmune disease |                |                  |                |                  |
| No              | 1.00 Reference |                  | 1.00 Reference |                  |
| Yes             | 1.22 (0.89–1.66) 0.2179 |                  | 1.26 (0.92–1.72) 0.1469 |
| Allergic disease |                |                  |                |                  |
| No              | 1.00 Reference |                  | 1.00 Reference |                  |
| Yes             | 1.27 (1.17–1.37) <0.0001 |                  | 1.22 (1.12–1.32) <0.0001 |
| Cancer          |                |                  |                |                  |
| No              | 1.00 Reference |                  | 1.00 Reference |                  |
| Yes             | 0.45 (0.23–0.9) 0.0242 |                  | 0.53 (0.27–1.07) 0.0753 |

HR = hazard ratio, CI = confidence interval. Adjusted HR: adjusted for inflammatory disease of cervix, vagina, and vulva, age and comorbidity in Cox proportional hazards regression.
TABLE 3. Incidence Rate and HR of Endometriosis in Patients With Inflammatory Diseases of the Cervix, Vagina, and Vulva

| Variables                  | Endometriosis (No. N = 79512) | Endometriosis (Yes N = 79512) | Crude HR (95% CI) | Adjusted HR (95% CI) |
|----------------------------|-------------------------------|-------------------------------|-------------------|---------------------|
| Age, y (means ± SD)        |                               |                               |                   |                     |
| 20–29                      | 632                           | 250,129                       | 2.53              | 2.03 (1.92–2.13)    |
| 30–39                      | 893                           | 235,489                       | 3.79              | 2.13 (1.97–2.30)    |
| 40–49                      | 473                           | 183,294                       | 2.58              | 2.09 (1.87–2.33)    |
| 50–55                      | 11                            | 43,917                        | 0.25              | 2.01 (1.92–2.12)    |
| Comorbidity infertility    |                               |                               |                   |                     |
| No                         | 1977                          | 708,707                       | 2.79              | 2.08 (1.97–2.19)    |
| Yes                        | 32                            | 4123                          | 7.76              | 1.14 (0.77–1.69)    |
| Leiomyoma of uterus        |                               |                               |                   |                     |
| No                         | 1916                          | 702,777                       | 2.73              | 0.20 (1.96–2.19)    |
| Yes                        | 93                            | 10,053                        | 9.25              | 2.04 (1.94–2.16)    |
| Autoimmune disease         |                               |                               |                   |                     |
| No                         | 1994                          | 709,279                       | 2.81              | 2.08 (1.97–2.19)    |
| Yes                        | 15                            | 3551                          | 4.22              | 1.35 (1.06–1.71)    |
| Allergic disease           |                               |                               |                   |                     |
| No                         | 1833                          | 662,544                       | 2.77              | 2.07 (1.96–2.19)    |
| Yes                        | 176                           | 50286                         | 3.50              | 2.02 (1.91–2.13)    |
| Cancer                     |                               |                               |                   |                     |
| No                         | 2007                          | 710,818                       | 2.82              | 2.07 (1.96–2.18)    |
| Yes                        | 2                             | 2012                          | 0.99              | 2.97 (1.60–14.73)   |

CI = confidence interval, HR = hazard ratio, IR = incidence rate per 1000 person-years. Adjusted HR: adjusted for inflammatory disease of cervix, vagina, and vulva, age and comorbidity in Cox proportional hazards regression.

*P < 0.05.  ***P < 0.001.

DISCUSSION

This is the first nationwide population-based study to investigate whether lower genital tract infection exhibited an increased risk of endometriosis. To avoid selection bias, we included subjects with at least 3 ambulatory claims or at least 1 in-patient claim among the patients with inflammatory diseases of the cervix, vagina, or vulva. We exclude patients with endometriosis before index date, patients younger than 20 years, and those older than 55 years. All the participants were assigned a unique PIN number and could be traced through NHIRD during the study period. Compared with the control cohort, endometriosis was significantly higher for patients with inflammatory diseases of the cervix, vagina, and vulva (HR = 2.01, 95% CI = 1.91–2.12). Moreover, we demonstrated that the incidence of endometriosis was 5.83 per 1000 person-years among patients with inflammatory diseases of the cervix, vagina, and vulva patients without comorbidity such as infertility (HR = 2.02, 95% CI = 1.92–2.13), leiomyoma of uterus (HR = 2.04; 95% CI = 1.94–2.16), autoimmune diseases (HR = 2.01; 95% CI = 1.91–2.12), allergic diseases (HR = 2.02; 95% CI = 1.91–2.13), and cancer (HR = 2.01; 95% CI = 1.90–2.12). Therefore, lower genital tract infection increased the risk of endometriosis.

Alternating concentrations of estradiol and progesterone modulate the growth of the human endometrium during the menstrual cycle. The varying concentrations of these female sex hormones have significant effects on the development of infections. For example, progesterone inhibits lymphocyte proliferation in the uterus, which increases the likelihood of a bacterial infection. Conversely, estrogen deficiencies play an important role in the development of urinary tract infections. A higher concentration of estradiol attracts more macrophages, which infiltrate the endometrium to help fight bacterial infections. Therefore, lower genital tract infection increased the risk of endometriosis.

There are considerable evidences indicating that the estrogen aggravates Chlamydia trachomatis and Mycoplasma genitalium infections, which may increase the risk in developing endometriosis. Endometriosis is common in reproductive-age female. Estrogen concentration is gradually lower until finally menopause. Most women have menopause between 45 and 55 years. In this study, we included patients with inflammatory diseases of cervix, vagina and vulva between age 20 and
55 years. Patients at age between 30 and 39 years had the highest risk (1.57, 1.48–1.66) in having endometriosis, whereas age between 40 and 49 years exhibited the same risk (1, 0.94–1.08) comparing with patients at age between 20 and 29 years. Patients at age between 50 and 55 years showed protective (0.18, 0.14-0.23) against endometriosis comparing to patients at age between 20 and 29 years. Estrogen is at lower concentration in women older than 40 years, hence lessens the inflammatory responses induced by infections, which lower the risk of endometriosis.

Endometriosis is identified as a hormone-dependent inflammatory disease because its symptoms are enhanced by estrogen and progesterone. The growth of an endometriotic lesion is predominately enhanced by the effect of estradiol on the estrogen receptor $\alpha$, which can subsequently increase lesion size, fluid volume, and epithelial proliferation. $^{29}$ Estrogen could also increase the expression levels of vascular endothelial cell growth factor (VEGF), in both ectopic and eutopic endometrium, to sustain the progression of endometriosis. $^{30–33}$ In addition to estrogen, bacterial endotoxins, such as lipopolysaccharide, could also enhance the growth of eutopic and ectopic endometriotic tissue. Previous studies have shown that bacterial endotoxins significantly increase the expression levels of hepatocyte growth factor, VEGF, IL-6, IL-8, and TNF-$\alpha$ in both endometrial stromal cells and macrophages. $^{34,35}$ The proliferation of endometrial stromal cells has also been shown to be significantly increased when treated with combined estradiol and lipopolysaccharide. $^{36}$ In addition, the secretion of inflammatory cytokines, such as IL-6 and TNF-$\alpha$, has been found to be higher in macrophages isolated from endometriosis patients. $^{37}$ The aforementioned findings suggest that an alteration in female sex hormones could increase the risk of bacterial infection and enhance the growth of ectopic and eutopic endometriotic tissues, which worsen endometriosis.

Lactobacillus species are the main bacterium species found in the healthy female vagina; however, some healthy women may exhibit microbiota without a significant amount of Lactobacillus species. $^{38–45}$ Ravel et al$^{46}$ found that 27% of women, among 396 asymptomatic sexually active women with vaginal pH $>4.5$, had higher amounts of anaerobic bacteria including: Prevotella, Dialister, Atoptohium, Gardenerella, Meganphaera, Peptoniphilus, Sneathia, Eggerthella, Aerococcus, Fingoldia, and Mobiluncus compared with women with vaginal pH $<4.5$. Indeed, the level of microbiota in the human vagina is highly dynamic, whereby Lactobacillus concentrations can change during the course of the menstrual cycle. $^{47}$ Brotman et al$^{42}$ show rapid changes in microbiota, which could lead to a temporary vaginal infection with spontaneous recovery. As such, differences in vaginal pH and microbiota may

| Variable | Adjusted HR (95% CI) |
|----------|---------------------|
| Infertility |                   |
| No No | 1 (Reference) |
| No Yes | 2.54 (1.79–3.60)$^{***}$ |
| Yes No | 2.02 (1.92–2.14)$^{***}$ |
| Yes Yes | 2.88 (2.36–3.50)$^{***}$ |
| Inflammatory diseases of cervix, vagina, and vulva | Infertility |
| No No | 1 (Reference) |
| No Yes | 3.67 (2.98–4.52)$^{***}$ |
| Yes No | 2.05 (1.94–2.16)$^{***}$ |
| Yes Yes | 4.90 (4.28–5.60)$^{***}$ |
| Leiomyma of uterus | Autoimmune disease |
| No No | 1 (Reference) |
| No Yes | 1.52 (0.92–2.53) |
| Yes No | 2.01 (1.91–2.12)$^{***}$ |
| Yes Yes | 2.15 (1.45–3.19)$^{***}$ |
| Allergic disease | Cancer |
| No No | 1 (Reference) |
| No Yes | 1.23 (1.06–1.44)$^{**}$ |
| Yes No | 2.02 (1.91–2.13)$^{***}$ |
| Yes Yes | 2.35 (2.13–2.60)$^{***}$ |
| Cancer |                |
| No No | 1 (Reference) |
| No Yes | 0.36 (0.09–1.44) |
| Yes No | 2.01 (1.90–2.12)$^{***}$ |
| Yes Yes | 1.07 (0.48–2.38) |

CI = confidence interval, HR = hazard ratio, IR = incidence rates, per 1000 person-years.

$^{***} P < 0.001$.

$^{**} P < 0.01$.

$^{*} P < 0.05$.

$^{\dagger}$ Adjusted for inflammatory disease of cervix, vagina, and vulva, age and comorbidity in Cox proportional hazards regression.

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increase the possibility of lower genital tract infections and the associated risk of endometriosis. Lower genital tract infections may also increase the risk of subclinical pelvic inflammatory disease, which is another common risk factor for endometriosis.

In conclusion, this nationwide study of 79,512 patients with lower genital tract infection over 738,648 follow-up person-years indicates that these patients have a 2.01-fold increased risk of endometriosis when compared with the general population. As such, our results demonstrate that genital tract inflammation/infections increase the risk of endometriosis. These findings provide the importance of treating lower genital tract infection for preventing endometriosis.

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