The prevalence and risk factors for the most frequent lower genital tract infections among adolescents and young females

Theses of doctoral (Ph.D.) dissertation

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1. Introduction

Genital *Chlamydia trachomatis* infection is of public health importance due to the well documented late consequences of the upper genital tract. Early diagnosis is a prerequisite for the prevention of salpingitis, tubal damage, ectopic pregnancy and chronic pelvic pain in females. The increasing incidence of ectopic pregnancies in many parts of the world is an indirect marker of prior and present high numbers of undiagnosed pelvic inflammatory disease cases.

Since overwhelming majority of chlamydial infections are asymptomatic in their character, sampling of potentially infected persons is based on the presence of risk factors of genital chlamydial infection. Young age is the strongest risk factor of infection as the prevalence of genital chlamydial infection is the highest among those younger than 20 years, and decreases in later ages.

Domeika et al presented data on *C. trachomatis* infections in Eastern Europe, but the prevalence and determinants of chlamydia is still hardly known in the region.

The most frequent vaginal infection is bacterial vaginosis, which is asymptomatic in the majority of cases. It may have gynecologic and obstetric complications also. Bacterial vaginosis may cause ascending infection, pelvic inflammatory disease, spontenous abortion and premature delivery.

2. Objective

2.1. The epidemiology of genital *Chlamydia trachomatis* infection

Our objective was to investigate the prevalence of chlamydia among a young female population in Hungary, a country that has not been systematically studied regarding *C. trachomatis* prevalence. A second purpose of the present study was to evaluate the association between genital chlamydial infection and behavioral, historical and demographic correlates. In addition, a possible association between endocervical chlamydia infection and vaginal infection was also tested as a biological marker for the presence of *C. trachomatis* infection.

1. The first aim of the study was to determine the prevalence of endocervical chlamydia infection at the adolescent gynecology outpatient clinic of the First Department of Obstetrics and Gynecology of Semmelweis University.

2. A second purpose of the present study was to evaluate the association between genital chlamydial infection and behavioral, historical and demographic correlates.
3. In addition, a possible association between endocervical chlamydia infection and vaginal infection was also tested as a biological marker for the presence of \textit{C. trachomatis} infection.

4. Another purpose was to evaluate the role of 20 or more polymorphonuclear leukocytes per high-power microscopic field of endocervical smear in predicting cervical \textit{Chlamydia trachomatis} infection.

5. The next aim was to determine the number and rate of ectopic pregnancies according to the published statistical data.

6. The next objective was to investigate the clinical and histopathological correlations of immunoreactivity to \textit{Chlamydia trachomatis} and to epitopes of the \textit{C. trachomatis} 60 kDa heat shock protein among women with ectopic pregnancy.

\textbf{2.2. The most prevalent vaginal infections}

1. Our aim was to determine the prevalence of vaginal infections among patient presenting for induced abortion of social reason with special regard to bacterial vaginosis.

2. My objective was to evaluate the effectivity of topical boric acid as a maintenance therapy in the prevention of relapses of recurrent vulvovaginal candidosis among non-pregnant women.

\textbf{3. Methods}

\textbf{3.1. The epidemiology of genital \textit{Chlamydia trachomatis} infection}

Between January and December 2003, 408 consecutive, unselected, sexually active nonpregnant women aged 16-24 years were enrolled in the study from all over Hungary, with their written consent. There was no any client who declined to participate. Young women who underwent antibiotic treatment in the previous 4 weeks were not considered for enrollment. The study was approved by the Ethical Committee of the Semmelweis University.

Each participant completed a self-administered and anonymous questionnaire containing questions about their sexual behavior, historical parameters, method of contraception, and symptoms of vaginitis (including vaginal discharge, vaginal itching or burning, and a fishy odour). Sexual history, behavioral parameters and possible symptoms of the last sexual partner were also recorded.
The study site was the gynecology outpatient clinic of the First Department of Obstetrics and Gynecology of Semmelweis University, Budapest, Hungary. This outpatient clinic admits patients from all over Hungary without referrals from their general practitioners. A standardized gynecologic examination was done, including a colposcopy of the lower genital tract, oncocytoology and bimanual pelvic examination. For each participant in the study, a sampling of the uterine cervix was taken following speculum inspection. *C. trachomatis* was identified using a polymerase chain reaction (Amplicor, PCR CT kit, Roche Diagnostic System Inc., Canada) according to the instructions of the manufacturer.

Vaginal microbial disorder was evaluated by vaginal pH, potassium-hydroxide test, and light microscopy of vaginal fluid samples. Vaginal pH was measured by pH indicator paper. Vaginal fluid samples were placed on slides, air dried, and stained with methylene blue. Amsel’s criteria were used for making a diagnosis of bacterial vaginosis. Vaginal candidiasis was diagnosed by the presence of yeast elements, and confirmed by culture on Sabouraud’s medium. The diagnosis of Trichomonas vaginitis was made on wet-mount slide. The criteria for making a diagnosis of cytolytic vaginosis was the presence of cytolysis of vaginal epithelial cells, without any other pathogen seen in the slide. A slide containing lactobacilli, vaginal epithelial cells without sign of cytolysis and absence of polymorphonuclear granulocytes was diagnosed as no vaginitis.

For patients diagnosed with an endocervical *C. trachomatis* infection, a single oral dose of 1000 mg azithromycin was administered to them and to their partners.

Based on the published statistical data in Hungary, between the years of 1931 and 2006, we investigated the the number and rate of ectopic pregnancies. Data of reported pregnancies were obtained from the National Institute of Statistics and the Hungarian College of Obstetricians and Gynecologists. Incidence of ectopic pregnancies was calculated as rates per 1000 live births and per 1000 reported pregnancies including live births, legally induced abortions, miscarriages and ectopic pregnancies.

We investigated the clinical and histopathological correlations of immunoreactivity to *Chlamydia trachomatis* and to epitopes of the *C. trachomatis* 60 kDa heat shock protein among women with ectopic pregnancy in association with histological diagnosis of plasma cell salpingitis, with peritubal pelvic adhesions and previous pelvic inflammatory disease.

### 3.2. The most prevalent vaginal infections
In the framework of the study performed between 1st January 2007 and 31st May 2007 vaginal swabs taken from patients presenting for induced abortion of social reason were
examined by microscopy. Samples were taken by colposcopy from the anterior and lateral fornix of the vagina in the morning of the clinical admission from 298 pregnant women whose gestational age were under 12 weeks.

Between January 2000 and December 2002 we used topical boric acid as a maintenance therapy in the prevention of relapses of recurrent vulvovaginal candidosis among 35 non-pregnant women. The participants were above the age of 18, who had four or more previous episodes of proved fungal vaginitis during a 12 month period. After treating the acute infection with 1 x 150 mg oral fluconazole, we started the maintenance regimen with topical boric acid. The efficacy of this prophylactic therapy was measured with the occurrence of acute fungal vaginitis during the period of maintenance therapy and 12 month after. The study site was the gynecology outpatient clinic of the First Department of Obstetrics and Gynecology of Semmelweis University, Budapest, Hungary.

3.3. Statistical analysis
An analysis of chlamydia infections using categorical variables was performed using a chi-square test. A p-value of less than 0.05 was considered statistically significant. Odds-ratios (OR) and 95% confidence intervals (95% CI) were used to determine statistical significance and student t-test was used to evaluate the difference of age at first sexual intercourse between groups.

4. Results

4.1. The epidemiology of genital *Chlamydia trachomatis* infection

4.1.1. Prevalence
The prevalence of chlamydial infection in the study population was 7% (27/387). The prevalence of endocervical *C. trachomatis* infection was compared between those with and without signs and symptoms of lower genital tract infection. The rate of infection was 10,1% (17/169) among those seeking gynecologic care for symptoms of lower genital tract infection. In contrast, the prevalence of infection was 4,6% (10/218) in asymptomatic patients (p=0,036; OR: 2,33; 95% CI: 0,98-5,63).

4.1.2. Demographic, historical and behavioral parameters
Marital status and schooling were not associated with endocervical chlamydia finding nor was previous gravidity.
Mean age at first sexual intercourse (coitarche) was 16.5 years in patients infected by *C. trachomatis*, and 17.2 years among those not infected (p=0.032). The total number of lifetime sexual partners was higher among chlamydia infected young women (6.04) compared to those not infected (3.4). Women who had at least three life-time partners were more likely to have *C. trachomatis* in the cervix compared to those who had two or less partners (p<0.001; OR: 22.25; 95% CI: 3.56-917.47). The number of sexual partners in the last three months was not related to the endocervical chlamydia finding and a new sexual partner in the last three months did not increase significantly the likelihood of *C. trachomatis* infection. The prevalence of endocervical infection was 10.6% among those with a new partner as compared to 6.2% in those who had not had a new partner. Smoking was not associated with endocervical chlamydial infection.

The prevalence of endocervical *C. trachomatis* infection was 9.1% among patients who were not using contraceptive methods as compared to 7.5% of those using oral contraceptives (OC), and 5.3% using condoms. Interestingly, patients who used both OC and condoms had the highest prevalence of endocervical infection (11.8%). Although the prevalence of chlamydia was lower among those using condoms as compared to those using no form of contraception, this difference was not statistically significant (p=0.28).

Previously diagnosed sexually transmitted infection of the subjects or their sexual partners did not influence the rate of endocervical *C. trachomatis* infection.

Urethral discharge in the sexual partner was associated with endocervical chlamydia being significantly more common among those infected than not infected (p=0.019; OR: 7.38; 95% CI: 1.11-36.77).

### 4.1.3. Vaginal coinfection

In patients with a positive endocervical chlamydia finding, the prevalence of bacterial vaginosis was significantly higher (33.3%) compared to those not harboring chlamydia in the cervix (11.6%) (p<0.005; OR: 3.79; 95% CI: 1.46-9.63). The prevalences of vaginal candidiasis and trichomoniasis were similar among those with and without endocervical chlamydial infection. The frequency of vaginitis was also different among the groups of *C. trachomatis* positive and negative patients. Overall, 55.6% of patients with *C. trachomatis* infection had co-existing vaginal disorder compared to 30% of the chlamydia negative patients (p<0.01; OR: 2.92; 95% CI: 1.24-6.9). Among patients with bacterial vaginosis the rate of endocervical chlamydial infection was significantly higher (17.6%) than among those without vaginal disorder (4.5%) (p<0.005; OR: 4.5; 95% CI: 1.63-12.4).
4.1.4. Mucopurulent cervicitis

20 or more polymorphonuclear leukocytes per high-power microscopic field of endocervical smear was associated with cervical Chlamydia trachomatis infection (p=0.01; OR: 2.72; 95% CI: 1.16-6.41). The rate of the microscopic diagnosis of mucopurulent cervicitis was 30% (116/387). Beside the 7% prevalence of chlamydia, the sensitivity, specificity and positive predictive value for polymorphonuclear cells to predict the presence of C. trachomatis were: 51.8%, 71.7%, and 12.1% respectively. According to these results, 20 or more polymorphonuclear leukocytes per high-power microscopic field of endocervical smear indicates further evaluation of the etiology.

4.1.5. Ectopic pregnancy, as late consequence of pelvic inflammatory disease, in Hungary (1931-2006)

The incidence and rate of ectopic pregnancies were calculated per 1000 live births and per 1000 reported pregnancies between 1931 and 2006.

From 1931, when national surveillance for pregnancies began in Hungary, to 2006, the rate per 1000 reported live births tripled from 3.4 to 12.2. From the first (1931-1940) to last decade (1996-2006) of the period studied, the average rate of ectopic pregnancies per live births changed from 1:208 to 1:79.

Similarly, the rate of ectopic pregnancies per 1000 reported pregnancies increased from 3.7 to 7.5 during these decades. From the first (1931-1940) to last decade (1996-2006) of the period studied, the average rate of ectopic pregnancies per reported pregnancies changed from 1:241 to 1:140.

4.1.6. Serologic responses of patients with ectopic pregnancy to epitopes of the Chlamydia trachomatis 60 kDa heat shock protein

We analysed the role of 60 kDa heat shock protein mediated immune mechanisms in the etiology of tubal damage leading to ectopic pregnancy.

The women with ectopic pregnancies were more likely to be unmarried (22.4% vs 6.7%; p<0.04), have a prior history of ectopic pregnancy (17.9% vs 0; p<0.04), or to have had at least one prior episode of medical treatment for pelvic inflammatory disease (29.4% vs 20%; p<0.05) than did controls. Patients with ectopic pregnancies were also more likely than their intrauterine pregnant controls to have present anti-chlamydial immunoglobulin G (52.2% vs 26.7%; p<0.005). For most of the epitopes the prevalence of antibodies were significantly
greater in the ectopic patients. Among the ectopic pregnancy group, antibodies to only two of the heat shock protein epitopes were more prevalent in women with histological diagnosis of plasma cell salpingitis, peritubal pelvic adhesions and previous pelvic inflammatory disease. A total of 62.7% of the ectopic pregnancy patients was positive for at least one indicator of a pelvic infection: histological salpingitis, pelvic adhesions and/or a history of pelvic inflammatory disease. Among ectopic pregnancy patients who were positive for antibodies to C. trachomatis surface components and also for antibodies to at least one of the heat shock protein epitopes had a higher prevalence than the antibody-negative patients in terms of histological salpingitis (66.7% vs 21.7%; p=0.002), pelvic adhesions (59.3% vs 13%; p=0.0002) and past history of pelvic inflammatory disease (55.6% vs 21.7%; p=0.01).

4.2. The most prevalent vaginal infections

4.2.1. The prevalence of bacterial vaginosis among females admitted for induced abortion

The prevalence of bacterial vaginosis was 23% (68/298). Among participants below 25 years the prevalence was significantly greater (30.6%) than among patients above (18.2%) (p=0.01; OR: 1.99; 95% CI: 1.11-3.57). In the study the prevalence of the postoperative pelvic inflammation was only 1.3% (4/298), lower than the results published in the literature. None of the patients with pelvic inflammatory-like complaints belonged to the bacterial vaginosis group. The late complications of the possible post-abortional appearing upper genital tract infections can basically influence the later fertility, that is the reason why we have to lay emphasis upon prevention.

4.2.2. The maintenance therapy of chronic recurrent vulvovaginal candidosis with topical boric acid

Four patients of the 35 were excluded from the study because of interruption of the maintenance therapy. During the period of the maintenance therapy recurrent infection did not occure among 26 (83.9%) patients of the remaining 31, although 5 patients experienced symptomatic fungal vaginitis. After completing the 12 months period of topical boric acid prophylaxis, one year long follow-up was carried out. During this follow-up period 11 (35.5%) of 31 patients had acute fungal episode, the remaining 20 (64.5%) did not experience symptomatic infection. Side effects were not mentioned during the study.

The follow-up study of patients with chronic recurrent vulvovaginal candidosis proved the efficacy of topical boric acid therapy.
5. Conclusions

Concerning generalizability of our clinical data to the wider population of Hungarian women, the study population involved participants with both metropolitan and rural domiciles. According to my data, the prevalence of *Chlamydia trachomatis* endocervicitis has not changed during the last two decades in Hungary. The results of my study are in accordance with the data reported in other countries. The tasks for the prevention of salpingitis, tubal damage, ectopic pregnancy and chronic pelvic pain in young females are as follows.

1. Since overwhelming majority of chlamydial infections are asymptomatic in their character, sampling of potentially infected persons is based on the presence of risk factors of genital chlamydial infection. Risk factors might help to select those women in whom testing for endocervical *C. trachomatis* infection with PCR is suggested.

2. The risk factors for genital chlamydial infection below 25 years of age in Hungary:
   - Younger age (16 or below) at first sexual intercourse
   - More than three life-time sexual partners
   - Urethral discharge of the sexual partner
   - Signs and symptoms of lower genital tract infection
   - Bacterial vaginosis
   - Two or more sexual partners in the preceding 3 months in the group of participants below 20 years of age

3. According to my results, the diagnosis of bacterial vaginosis among sexually active young females should rise the suspicion of higher occurrence of *Chlamydia trachomatis* cervicitis, and indicates chlamydia testing.

4. Overall, most of the patients with *C. trachomatis* infection had co-existing vaginal disorder, therefore among females with positive chlamydia result, the vaginal coinfections should be excluded.

5. The microscopic diagnosis of mucopurulent cervicitis indicates further bacteriologic tests (PCR) to identify the pathogen (*C. trachomatis, Neisseria gonorrhoeae, Ureaplasma urealyticum, Mycoplasma genitalium*).

6. The increasing incidence and rate of ectopic pregnancies is an indirect marker of increasing numbers of undiagnosed pelvic inflammatory disease cases.
7. Our data strongly suggests that a prior or prolonged *C. trachomatis* infection may be necessary for the induction of heat shock protein related tubal immunopathology. Immune sensitisation of the host to specific regions of the chlamydial 60 kDa heat shock protein might be responsible for initiating a delayed hypersensitivity reaction within the Fallopian tubes, resulting in tubal damage or peritubal pelvic adhesions. *Chlamydia trachomatis* is certainly not the only cause of ectopic pregnancy, as demonstrated by the absence of immunity to this organism in 34.3% of ectopic pregnancies.

8. Among females below 25 years of age admitted for induced abortion of social reason the prevalence of bacterial vaginosis was 30%. The prevalence of bacterial vaginosis was significantly higher among women younger than 25 years compared to those above. Among females younger than 25 years presenting for induced abortion the diagnosis of bacterial vaginosis indicates further cervical bacteriologic tests.

9. In our study the incidence of the postoperative pelvic inflammatory disease after artificial abortion was only 1.3% (4/298). According to our data the universal perioperative antibiotic prophylaxis is not necessary.

10. Topical boric acid should be the first choice as a maintenance therapy in the prevention of relapses of recurrent vulvovaginal candidosis among non-pregnant women. During the first three months, the patients should use the vaginal suppository containing boric acid weekly.
6. Publication list

6.1. Publications in the subject of the dissertation


**IF: 3,650**


**IF:** 3,669

**IF:** 2,71

15. **Ujházy A**, Csaba Á, Bőze T, Derzsy Z, Sziller I, Rigó J. Az endocervicalis Chlamydia trachomatis fertőzés fiatal nők között hazánkban. STD és Genitális Infektológia (accepted for publication)

### 6.2. Publications written in other subjects


