Chlamydia trachomatis and cervical intraepithelial neoplasia in married women in a Middle Eastern community

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الكلاميديا التـراخومية والأورام داخل البطانية في عنق الرحم لدى المتزوجات في إحدى المجتمعات الشرق أوسطية

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الخلاصـة: إن الغرض المتوخَّى من هذه الدراسة هو التعرُّف على مدى الترابط بين العدوى الفرجية بالكلاميديا وبين الأورام دخل البطانية في عنق الرحم. وقد جمع الباحثون المعطيات ضمن دراسة للحالات والشواهد شملت 60 مريضة بورم داخل بطاني في عنق الرحم شخصت إصابتهن بالخزعة، إلى جانب 85 إمرأة كان تنظير عنق الرحم والخزعة منه سوياً لديهن. وقد لاحظ الباحثون أن الأضداد المصلية للكلاميديا التـراخومية تتصاحب مع ازدياد خطر الإصابة بالورم داخل البطاني في عنق الرحم (وكان معدل الأرجحية 7.3، وكانت فتـرة الثقة 95٪، إذ تراوحت النتائج بين 1.5 و35.2). وكان هناك ترابط يُعْتَدُّ به إحصائياً بين وجود أضداد مشتملة للكلاميديا التـراخومية وبين الورم داخل البطاني في عنق الرحم (فكان معدل الأرجحية 5.5، وكانت فتـرة الثقة 95٪، إذ تراوحت النتائج بين 2.4 و12.4). وتشير هذه النتائج إلى ترابط قوي بين الأورام داخل البطانية في عنق الرحم وبين التهاب عنق الرحم بالكلاميديات.

ABSTRACT The objective of this study was to determine the association between vaginal *Chlamydia* infection and cervical intraepithelial neoplasia (CIN). Data were collected in a case–control study for 60 patients with CIN in biopsy and 85 control subjects with normal colposcopy and biopsy. Serum antibodies to *C. trachomatis* were associated with an increased risk for CIN [odds ratio (OR) = 7.3; 95% confidence interval (CI) 1.5–35.2)]. There was also a significant association between presence of inclusion bodies for *C. trachomatis* and CIN (OR = 5.5; 95% CI 2.4–12.4). These results indicate a strong association between CIN and chlamydial cervicitis.

Les infections à *Chlamydia trachomatis* et la néoplasie cervicale intraépithéliale chez les femmes mariées d'une communauté du Moyen-Orient

RÉSUMÉ L'objectif de cette étude était de déterminer la relation entre les infections vaginales à *Chlamydia* et la néoplasie cervicale intraépithéliale (CIN). Des données ont été collectées lors d'une étude cas-témoin portant sur 60 patientes dont la biopsie confirmait une CIN et un groupe témoin de 85 personnes dont la colposcopie et la biopsie étaient normales. Les anticorps sériques anti-*C. trachomatis* étaient associés à une augmentation du risque de CIN (*odds ratio* = 7,3 ; intervalle de confiance (IC) 95 % 1,5 – 35,2)]. Une association significative a également été observée entre la présence de corps d'inclusion de *C. trachomatis* et la CIN (*odds ratio*= 5,5 ; IC 95 % 2,4 – 12,4). Ces résultats indiquent une association importante entre la CIN et une cervicite à *Chlamydia*.

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Introduction

Infection with *Chlamydia trachomatis*, a highly prevalent sexually transmitted agent worldwide, is mostly asymptomatic (70%–80%) and often remains undetected. Besides causing cervicitis and urethritis, infection may result in serious secondary complications such as pelvic inflammatory disease and pelvic pain, tubal infertility and ectopic pregnancy [*1*]. In addition, *C. trachomatis* has been suggested to be a cofactor in the development of cervical cancer [*2,3*]. However, in some studies no association was found between *C. trachomatis* and cervical neoplasia [*4*].

The objective of this study was to determine the association between vaginal chlamydial infection and cervical intraepithelial neoplasia (CIN).

Methods

For this retrospective case–control study, participants were selected from the total of 609 women visiting the gynaecology clinic in our hospital between January 2002 and May 2003 and who were referred to the colposcopy clinic at Mirza Koochak Khan Hospital, Tehran. Colposcopy showed suspicious/abnormal cervix in 200 women and biopsy was taken for them. Since many of the patients had been referred from other cities, 20 patients were not available. From the 180 remaining, we selected 60 patients with CIN in biopsy and 85 control subjects with normal colposcopy and biopsy after statistically matching for age and social status. We used the Bethesda system for classification of CIN.

Each participant was tested for *C. trachomatis* using 2 methods: 1) an immunuofluorescence technique to measure specific antichlamydial IgG-antibody in blood samples (titres of ≥ 1/64 were considered positive for *C. trachomatis*); and 2) Giemsa staining for detecting inclusion bodies characteristic of *C. trachomatis*.

Each participant completed a detailed questionnaire administered by a trained interviewer covering demographic data, general health, obstetrical and gynaecological history and other risk factors. All participants gave written informed consent to write their participation.

Statistical methods

Categorical variables were summarized by absolute frequencies and percentages and were compared using chi-squared or Fisher exact test, as appropriate. In order to evaluate the association of *C. trachomatis* with CIN, both crude and Mantel–Hansel odds ratio (OR), adjusted for possible confounders, with 95% confidence interval, were calculated. For the statistical analysis *SPSS*, version 13.0 for Windows, was used. All *P*-values were 2-tailed, with statistical significance defined at $P \leq 0.05$.

Results

All participants were married and the age range was 20–65 years: 28 in the patient group and 33 in the control group were over 40 years old.

The overall prevalence of positive serum antibodies for *C. trachomatis* infection for cases and controls together was 26.2% (Table 1); 45.0% among women with CIN and 12.9% among controls, which was a highly significant difference ($P < 0.001$, unadjusted OR = 5.5). In addition, the prevalence of inclusion bodies for *C. trachomatis* in the CIN group was higher than in the control group (15.0% vs 2.4%, $P = 0.005$, unadjusted OR = 7.3). Participants negative for anti-*Chlamydia* antibody also had a negative evaluation for *C. trachomatis* inclusion bodies.

Of the CIN group and controls, 76.7% and 51.8% respectively had their first sexual contact at age ≤ 20 years, a statistically significant difference ($P = 0.002$). In addition, 30.0% of the women in the CIN group and 10.6%

Table 1 Association between CIN with the presence of IgG antibodies to *Chlamydia trachomatis* or inclusion bodies of *C. trachomatis*

Variable	Women with CIN ($n = 60$)		Controls ($n = 85$)		*P*-value	Crude OR (95% CI)
	No.	%	No.	%		
Positive *C. trachomatis* IgG	27	45.0	11	12.9	< 0.001	5.5 (2.4–12.4)
Presence of inclusion body	9	15.0	2	2.4	0.005	7.3 (1.5–35.2)
First sexual contact < 20 years	46	76.7	44	51.8	0.002	3.1 (1.4–6.3)
First pregnancy ≤ 16 years	18	30.0	9	10.6	0.003	3.6 (1.4–8.7)
Multiparity	36	60.0	35	41.2	0.02	2.1 (1.1–4.2)
History of abortion	31	51.7	25	29.4	0.007	2.6 (1.2–5.1)
Used OCP for ≥ 1 year	22	36.7	22	25.9	0.1	1.6 (0.8–3.3)
History of genital infection	42	70.0	42	49.4	0.01	2.4 (1.1–4.7)

CIN = cervical intraepithelial neoplasia; OR = odds ratio; CI = confidence interval.
OCP = oral contraceptive pills.

in the control group had their first pregnancy at age ≤ 16 years ($P = 0.003$), again a statistically significant difference. There was a history of genital tract infection in 70.0% of cases compared with 49.4% of controls ($P = 0.01$). Use of oral contraceptive pills for ≥ 1 year was not significantly different between the 2 groups ($P = 0.1$).

None of the women in this study had a history of cigarette smoking, therefore the association of smoking with CIN was not evaluated. Furthermore, we could not assess the relationship between CIN and number of sexual partners, because sexual relations outside marriage are punishable by law in the Islamic Republic of Iran, and this is why our specimen was taken from married women only.

Of the 60 women with CIN, 54 were diagnosed with CINI, 3 had CINII and 3 had CINIII. The prevalence of serum antibodies positive for *C. trachomatis* was 22 (41%) for CINI, 3 (100%) for CINII and 2 (66%) for CINIII.

The association of *C. trachomatis* and CIN remained significant after adjustment for first sexual contact < 20 years, first pregnancy ≤ 16 years, multiparity, history of abortion and genital infection (Table 2).

Table 2 Odds ratio (OR) for *Chlamydia trachomatis* infection in cases with CIN after adjustment for other risk factors

Risk factor adjusted for:	Adjusted OR (95% CI)
First sexual contact < 20 years	5.4 (2.3–12.7)
First pregnancy ≤ 16 years	5.0 (2.1–11.5)
Multiparity	5.1 (2.2–10.9)
History of abortion	4.5 (1.9–10.5)
History of genital infection	5.0 (2.2–11.1)

CIN = cervical intraepithelial neoplasia; CI =confidence interval.

Discussion

We found a significant association between CIN and the presence of *C. trachomatis* IgG (crude OR = 5.5) and the presence of inclusion bodies for *C. trachomatis* (crude OR = 7.3), which remained significant after adjusting for other risk factors. Schachter et al. also reported a significant excess of antibodies against *C. trachomatis* in women with cervical neoplasia compared to controls [5]. Moreover, Koskela et al. found that the presence of *C. trachomatis* antibodies was associated with an increased risk of cervical squamous cell carcinoma [6]. Wallin et al. reported a similar association in a Swedish study [7]. However, there are a number of studies with opposing results. In a study on 128 women with clinical signs of cervicitis, genital chlamydial infection did not directly influence the development of CIN [4].

Our study does provide information on this association in relation to this part of the world. To our knowledge, this is one of a few reports on the association between cervical abnormalities and *C. trachomatis* infection from the Middle East. However, when interpreting the results, many points need to be borne in mind:

- Ours is an exceptional population, in which the prevalence of both cervical abnormalities and *C. trachomatis* infection are low.
- Our sample included a greater number of older women compared with the majority of studies published in Western countries.
- This study only addressed the association between cervical abnormalities and *C. trachomatis* infection, and not between frank cancers and *C. trachomatis* infection.
- We had limited statistical power related to the number of cases of CINII/CINIII, leading us to combine them.
- Lastly, we did not evaluate human papilloma virus (HPV). It is possible that concurrent infection with HPV may exacerbate the effect of *Chlamydia* infection on cervical cells.

Discrepancies between our results and those from studies conducted elsewhere may be related to differences in the characteristics of the studied populations (such as age, patterns of sexual behaviour or openness in reporting sexual behaviour).

Our results show that chlamydial cervicitis is a strong risk factor for CIN. The link between bacterial infections and carcinogens is not clear, but genetic damage and neoplastic changes can be induced *in vitro* by co-culturing cells. Release of nitric oxide occurs in *C. trachomatis* infections. Recent evidence has also shown that *C. trachomatis* inhibits host cell apoptosis; these mechanisms could initiate or promote cervical carcinogens [8]. This study was a preliminary attempt to establish the prevalence of cervical abnormalities and chlamydial infection, and the association between the 2 in a low-prevalence, Middle Eastern population. Further research is needed to investigate the apparent association between CIN and *C. trachomatis* infection.

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Towards a strategy for cancer control in the Eastern Mediterranean Region

Towards a strategy for cancer control in the Eastern Mediterranean Region was developed in response to the increasing burden of cancer and the need for coordinated action in this regard. This publication reflects a shared commitment to reducing the incidence of cancer and improving the quality of life of those who develop cancer. By promoting an integrated approach to the provision of cancer control activities and services, it is hoped the publication will encourage and assist government and nongovernmental service providers to work more closely together in addressing cancer control:

Towards a strategy for cancer control in the Eastern Mediterranean Region is targeted at government and nongovernmental agencies whose work impacts on the delivery of cancer services and activities, as well as the wide range of individuals involved in the management and delivery of activities and services related to cancer and people affected by cancer.

The full text of this publication is freely available at: http://www.emro.who.int/dsaf/dsa1002.pdf