PREVALENCE OF *CHLAMYDIA TRACHOMATIS* IN CERVICAL AND VULVAR CARCINOMA OF THE LUBLIN REGION PATIENTS

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Abstract

The aim of the study was to evaluate frequency of occurrence of *Chlamydia trachomatis* infection in samples of cervical and vulvar cancer in patients of the Lublin Region. The study was performed on paraffin sections prepared from the specimens of cervical cancer obtained from 570 patients and of vulvar cancer from 46 patients. We identified archival diagnostic phase tissue specimens. The control material to that obtained from patients with cervical cancer consisted of normal cervical tissues. The control material to that obtained from patients with vulvar cancer were fragments of normal epithelial tissue collected from the same paraffin blocks containing material from the margin of surgical section during vulvectomy. In order to identify *Chlamydia trachomatis*, DNA isolated from archival material was analysed and PCR was performed using starters complementary to *Chlamydia trachomatis*. Statistically significantly higher frequency of the occurrence of *Chlamydia trachomatis* was observed in sections from patients with invasive cervical cancer compared to control group. In the analysed material, the frequency of cases of vulvar cancer with co-occurrence of *Chlamydia trachomatis* infection was not statistically significant.

*Chlamydia trachomatis* may not be directly involved in the oncogenic processes but may enhance the possibility of oncogenesis or infect cancer tissues opportunistically.

**Key words:** women, *Chlamydia trachomatis*, cervical cancer, vulvar cancer.

Recent studies of the aetiology of invasive cervical cancer (ICC) aim to identify factors that may influence susceptibility to progression of cervical neoplasia. The identification of cofactors is important because these factors may be amenable to the prevention. The progress of dysplastic lesions with participation of highly oncogenic HPV types is connected with the occurrence of additional factors promoting the process, such as infections including the ones caused by *Chlamydia trachomatis*. Other authors think that *Chlamydia trachomatis* is an independent factor, which might lead to pathological lesions of proto-oncogenic and oncogenic character within the cervix (11). Positive association between *Chlamydia trachomatis* microimmunofluorescence seropositivity and ICC have been found in a case-control study from England (OR=2.2) and a pooled analysis of cohort studies from Finland, Norway, and Sweden (OR=2.5) (1).

Vulvar cancer accounts for 5% of all female genital cancers and 1% of all malignancies in women (5). It has traditionally been considered a disease of older women, but in recent years the incidence among younger women has been increasing (5). Currently, the incidence has risen to 8%. Possible explanations for the increase in the incidence include longer life expectancy of the female population. Several studies have found the risk of vulvar cancer to be associated with low social class, cigarette smoking, early sexual initiation, and increasing number of sexual partners (3, 8).

A few recent studies of anogenital cancer have provided support for a long-held idea that sexually transmitted infections (STI), other than those due to HPV, such as *Chlamydia trachomatis* infections may be such HPV cofactors (1). Mechanistically, co-infection of these STIs with HPV could increase the risk of CIN progression by interfering with local immune response, by including paracrine and autocrine changes in the local cellular milieu or by simply causing direct tissue damage and thus increasing the likelihood that HPV infection and associated lesions persist and progress.

The purpose of the study was the evaluation of the occurrence of *Chlamydia trachomatis* co-infections detected in paraffin sections prepared from samples of cervical and vulvar cancer in women.
**Material and Methods**

The investigations were performed using formalin-fixed and paraffin-embedded cervical cancer specimens obtained from 570 women who were subjected to surgery during 1992-2002 at the Department of Gynaecological Surgery of the Medical University in Lublin because of histologically confirmed neoplastic lesions. The control group consisted of normal cervical tissues obtained from 50 patients that underwent myomectomy. There were no significant differences in mean age of women who underwent surgery due to planoeplithelial cervix cancer if compared to control women (44.93±13.49 vs 45.94± 6.12). The second group study was material consisted of paraffin embedded tissues collected from invasive vulvar cancer from patients who underwent surgery in Lublin Region hospitals in 1992-2002. The patients were from 33 to 88 years of age (mean age 73.3± 2.8). From each paraffin block, fragments were taken for PCR analysis. Eventually, tissues from 46 invasive vulvar cancer were qualified for the study group. From the same blocks, segments of normal vulvar epithelium were taken from the margin of surgical section during vulvectomy. These segments constituted the control group. The Ethical Committee of the University approved the study.

**DNA isolation.** Paraffin blocks were cut into 4 µm thick sections. The microtome was rinsed with alcohol before cutting each block. A new cutting blade was used for the cutting of each new block. The sections obtained in this manner were placed in a 1.5 ml testing tube with polypropylene and paraffin was removed in xylene at 37°C for 30 min. The deparaffinised tissues were homogenised in Hirt buffer (10 mM Tris-HCl, pH 7.5, 10 mM EDTA, 0.6% w/v SDS). The homogenate was incubated for 30 min at room temperature. Then, proteinase K was added to the final concentration of 50 µg/mL and homogenate was incubated for 24 h at 37°C. After the incubation was finished, half volume of isopropylene alcohol before cutting each block. A new cutting blade was used for the cutting of each new block. The sections obtained in this manner were placed in a 1.5 ml testing tube with polypropylene and paraffin was removed in xylene at 37°C for 30 min. The deparaffinised tissues were homogenised in Hirt buffer (10 mM Tris-HCl, pH 7.5, 10 mM EDTA, 0.6% w/v SDS). The homogenate was incubated for 30 min at room temperature. Then, proteinase K was added to the final concentration of 50 µg/mL and homogenate was incubated for 24 h at 37°C. After the incubation was finished, half volume of isopropylene alcohol and 0.1 volume of 3M acetate, pH 7.0, were added to the water phase obtained. The DNA samples obtained in this manner were then rinsed in 80% ethanol and dissolved in distilled water after drying. The samples with dissolved DNA were stored at -20°C. Quantitative determination of the DNA was carried out by the spectrophotometric method using an automatic spectrophotometer (Pharmacia Co.) In order to determine the amount of DNA in a given sample, 1 µl of the sample was dissolved in 69 µl of re-distilled water and placed in its measuring chamber. After automatic processing of the data measured, the result was read in µg/mL.

**Chlamydia trachomatis identification.** PCR II (nested-PCR) was used in order to identify bacterial deoxyribonucleic acid in the DNA isolated from the postoperative material. The product obtained in the first stage of PCR was used as matrix for PCR II. The size of the first stage product was 116 bp, and of the second stage product was 87 bp. The temperature for 7 individual stages of the II cycle of PCR II was: 95°C, 54°C, 72°C, 95°C, 54°C, 72°C, and 4°C, respectively. The time of duration of the individual stages was 4 min, 2 min, 1 min, 2 min, and 7 min. Thirty five cycles were performed. The time of duration of the whole programme was 3 h 55 min.

**Statistical analysis.** A method of statistical interference, verification of hypotheses based on homogeneity and independence test $\chi^2$, was used. Statistical significance was found at $P<0.05$. The statistical analyses were made on IBM PC, using SPSS 8.0 PL for Windows 95 and Statistica 5.0.

**Results**

*Chlamydia trachomatis* was identified in 26% (135/520) of the tissue sections from patients with squamous cell cervical carcinoma. In the control group, *Chlamydia trachomatis* organisms were observed in 4 cases (4/50), which constituted 8.0%. Statistically significantly higher frequency of *Chlamydia trachomatis* occurrence was observed in sections from patients with invasive cervical cancer compared to control without neoplastic lesions ($P<0.05$).

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Study group</th>
<th>C. trachomatis</th>
<th>Control group</th>
<th>C. trachomatis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive cervical cancer</td>
<td>n = 520</td>
<td>n =135 (26%)*</td>
<td>n = 50</td>
<td>n = 4 (8%)</td>
</tr>
<tr>
<td>Invasive vulvar cancer</td>
<td>n = 46</td>
<td>n = 3 (6.52%)</td>
<td>n = 46</td>
<td>n = 1 (2.1%)</td>
</tr>
</tbody>
</table>

n-number of patients

*- compared to control: $P<0.05$ ($P=0.012$)
The frequency of the occurrence of *Chlamydia trachomatis* in the analysed material from patients with invasive vulvar cancer was 6.52% (3/46) while it was 2.1% (1/46) in the control. The difference was not statistically significant. The Table 1 presents correlations between the frequency of occurrence of *Chlamydia trachomatis* infections in the study and control group.

**Discussion**

An analysis of the presence of *Chlamydia trachomatis* performed in the clinical study material revealed the chlamydial DNA in squamous cell cervical cancer in 26% of the studied samples. In comparison with 8% of the control group, the difference was statistically significant. The available data presented mainly by the Center for Disease Control in Atlanta and the Center for AIDS and Sexually Transmitted Disease in Seattle indicate that in women with intraepithelial neoplasia and with ICC, *Chlamydia trachomatis* infections are statistically significantly more frequently than in control group women, which is compatible with the results of our study (2).

*Chlamydia trachomatis*, as the promotional factor, may cause series of lesions leading to progression of cervical neoplasia. The organism is more and more frequently diagnosed as the reason of bacterial sexually transmitted diseases. In 1999, the infections with *Chlamydia* were found in 56,855 patients in the United Kingdom, which was a 61% increase compared to 1996 (6). In the United States, *Chlamydia trachomatis* infections are estimated to reach 700,000 cases a year (2), and in the world it is over 12 million (14).

Significantly higher frequency of the occurrence of *Chlamydia trachomatis* in high grade squamous intraepithelial neoplasia (H-SIL) and in ICC compared to our data was observed by Markowska (9) (respectively, 60% and 81% vs. 56.2% in control group). The infection was accompanied by an increase in the expression of growth and proliferation factors in cervical epithelium cells. These differences are probably due to the different population structure in Lublin and Poznan regions. Lublin is an agricultural region while Poznan is an industrial one.

Other authors also present differing data. Schloot *et al.* (12) using PCR method showed that there is high (40%) rate of the presence of *Chlamydia trachomatis* in cervical cancer tissues. However, Anttila *et al.* (1) demonstrated the presence of *Chlamydia trachomatis* DNA only in 5% of cases. The reason for this discrepancy is not known, but assay-specific methodological differences are a plausible explanation. Additionally, results of a study by Wallin *et al.* (13) indicated a relationship between *Chlamydia trachomatis* infection and cervical cancer development. Prospective analysis of cytological smears obtained in the course of screening examinations for cervical cancer in 1969-1995 in Sweden showed that the risk of cervical cancer development increases 17 times if HPV infection is accompanied by *Chlamydia trachomatis* infection. In turn, serological studies confirmed positive correlation between the level of *Chlamydia trachomatis* antibodies and the development of HPV-positive cervical cancer (1). Antibodies against *Chlamydia trachomatis*, including diverse serotypes, are identified predominantly in women having many sexual partners, as well as genital tract infections (4). In contrast to the above mentioned studies, Moscicki *et al.* (10) presented the data, which did not reveal any relationship between genital infection with *Chlamydia trachomatis* and the risk for the development of HSIL (high grade squamous intraepithelial lesion) associated with HPV infection. However, the study demonstrated the existence of a relationship between *Chlamydia trachomatis* and the risk for the development of LSIL (low grade squamous intraepithelial lesion). In conclusion, in our study we found statistically significantly higher frequency of the occurrence of *Chlamydia trachomatis* detected in histological sections prepared from ICC cases, compared to control.

There are only a few studies analysing co-infections in vulvar cancer. The ones available in the data bases (Medline, Pub Med) indicate higher rate of HSV-2 and *Chlamydia trachomatis* in the studied cases. Madeleine *et al.* (8) analysed specimens from 110 vulvar cancers for the presence of HSV-2 and *Chlamydia trachomatis* in the studied cases. Madeleine *et al.* (8) analysed specimens from 110 vulvar cancers for the presence of HSV-2 and *Chlamydia trachomatis* in the studied cases. In our study, we identified only 3 cases of HSV-2 and the presence of *Chlamydia trachomatis* in histological sections. These sections were collected from women below 50 years of age. In our previous studies evaluating the frequency of the occurrence of *Chlamydia trachomatis* and HSV-2 in vulvar smears using PCR, we estimated the occurrence of HSV-2 as 3%-4% (7).

In conclusion, in our study we found no correlation between the occurrence of *Chlamydia trachomatis* detected in histological sections prepared from vulvar carcinoma. *Chlamydia trachomatis* may not be directly involved in the oncogenic processes, but it may enhance the possibility of oncogenesis or infect cancer tissues opportunistically.

**References**


