Chlamydial Antibodies and Risk of Prostate Cancer

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Abstract

Objective: We assessed the risk of prostate cancer by exposure to Chlamydia trachomatis.

Method: Seven hundred thirty eight cases of prostate cancer and 2,271 matched controls were identified from three serum sample banks in Finland, Norway, and Sweden by linkage to the population based cancer registries.

Results: A statistically significant inverse association (odds ratio, 0.69; 95% confidence interval, 0.51-0.94) was found. It was consistent by different serotypes and there was a consistent dose-response relationship.

Conclusion: C. trachomatis infection is not likely to increase the risk of prostate cancer. Whether the inverse relationship is true or due to difficulties in measuring the true exposure in prostatic tissue by serology, confounders or other sources of error remain open. (Cancer Epidemiol Biomarkers Prev 2005;14(2):385–9)

Introduction

The prostate cancer is one of the most common malignant neoplasms in men all over the world. Annually, more than 10,000 men in the Nordic countries are diagnosed with prostatic cancer. Despite of its importance, relatively little is known of its aetiology. Long asymptomatic period and generally slow growth rate of the tumor have hampered the studies on the etiologic hypotheses.

Prostate cancer is rare below age 40, and then incidence rates double for each subsequent decade of life. Family history and ethnicity/country of residence are the only firmly established risk factors for prostate cancer (1). Besides hormones (2-4) and dietary factors (5-8), a role for sexual behavior and associated infectious agents has been supported by some previous studies of prostate cancer (1, 3, 9-11).

Chlamydia trachomatis is nowadays considered as the most common bacterial cause of sexually transmitted diseases (12). C. trachomatis is an obligate intracellular Gram-negative bacterium, which, like all chlamydial species, has a tendency to cause chronic persistent infections. In women, chronic C. trachomatis infections have been connected to pelvic inflammatory disease, ectopic pregnancies, and infertility (13-15). Furthermore, our earlier seroepidemiologic studies have suggested that chronic C. trachomatis infections increase the risk of cervical cancer (16-18). In sexually active men, C. trachomatis is the main cause of nongonococcal urethritis. Nonbacterial prostatitis, an idiopathic disease, is the most common form of prostate inflammation constituting about 50% of all cases. There has been much speculation, but only a little proof, that chlamydial infection might be responsible for many cases of apparent nonbacterial prostatitis (19, 20). However, as early as 1972, Mårdh et al. showed the more frequent presence of chlamydial antibodies in men with chronic prostatitis compared with the controls (21) and later studies have also suggested that antichlamydial immunoglobulin A (IgA) and immunoglobulin G (IgG) antibodies are present in patients with chronic prostatitis (22, 23).

The presence of C. trachomatis antibodies against several immunotypes and possibly also the presence of elevated titers against the agent may indicate the cumulative exposure to C. trachomatis infection (24, 25). There are also studies on the persistence of C. trachomatis antibodies with age and over time (24, 25). This is important because the risk factors for prostate carcinoma evidently exist at early phase of the disease preceding the long latency period in the development of cancer. Large serum sample banks stored in the Nordic countries enabled us to estimate the risk of prostate cancer among men with and without serologically diagnosed C. trachomatis infection by a longitudinal nested case-control design. The aim of our study was to evaluate the risk of prostate cancer due to infection with C. trachomatis.

Materials and Methods

Serum banks in Finland, Norway, and Sweden were used to study risk of prostate cancer in men with and without serologic diagnosis of C. trachomatis infection. More than 200,000 men have donated serum samples to the three serum banks participating in the study.

The Cohorts. The Finnish cohort contained blood samples of ~19,000 men aged 40 to 55 who participated in 1981 to 1982 in the first health examination for the Helsinki Heart Study, a primary-prevention trial in middle aged men to reduce coronary heart disease risk by lowering serum lipid levels with gemfibrozil (26). The participants were selected by screening from employees in two governmental agencies and five industrial companies. The serum samples were stored at −20°C. In Norway, the Janus project was initiated in 1973 and contained serum samples from about 160,000 men (27). Most participants were recruited from several counties in Norway.
during routine health examinations or in conjunction with screening for risk factors of cardiovascular diseases. The samples were collected also from blood donors from the Red Cross Blood Donor Center in Oslo and sera were stored at −25°C.

The men in The Northern Sweden Health and Disease Cohort were recruited through the Västerbotten intervention project (VIP), which started in Sweden 1985 and is still ongoing. Each year, all residents aged 30, 40, 50 and 60 were invited to participate in a health promoting project, including the donation of biological samples for future medical research. The participants were also recruited through the northern Sweden part of the WHO Multinational Monitoring of Trends and Determinants in Cardiovascular Disease Study (MONICA; refs. 28, 29). The MONICA study recruited participants in 1986, 1990, and 1994. These two projects, VIP and MONICA, in Västerbotten and Norrbotten consisted about 30,000 men as a representative population sample for the study. Plasma samples were stored at −80°C. All subjects provided informed consent before enrollment and the study was approved by each respective local Research Ethical Committee.

Cancer Registries. The Cancer Registry of Norway, the Finnish Cancer Registry, and the regional cancer registry at the Umeå Oncological Centre, which covers the four northernmost counties in Sweden, are population based and country- or county-wide (30). All the registries receive notifications from hospitals, hematology and pathology laboratories, and physicians and have almost 100% coverage in reporting. The registry practices and definitions are comparable due to close and long-lasting collaboration between the registries.

Case Ascertainment and Control Selection. Cases with prostate carcinoma were identified by linking the data files of the serum banks with the national cancer registries (regional in Sweden) in 1997 using personal identification numbers. Prostate cancers diagnosed before time of the blood sampling were excluded. When more than one pre-diagnostic sample was available, the earliest (the oldest) was chosen. For each case, four controls were selected randomly from those that were alive at the time of diagnosis of the case and without diagnosis of prostate cancer at end of 1996 and fulfilled the matching criteria: age at serum sampling (>2 years), time of serum sampling (<±2 months; in part of the Norwegian cohort, ±6 months), same country and region inside the country (Norway). If four controls per case fulfilling the matching criteria could not be identified, either matching criteria were widened or less than four control subjects were accepted. Some of the stored frozen Finnish samples had been accidentally thawed and were matched for thawing.

From the time of the serum sampling until end of 1996, 138 cases and 497 controls from Finland, 514 cases and 1,433 controls from Norway, and 86 cases and 341 controls from Sweden were available for analysis. Thus, the total number of cases and controls was 738 and 2,271, respectively.

Chlamydial Serology. C. trachomatis- and C. pneumoniae-specific IgG antibodies were measured by the microimmuno-fluorescence method, which is considered a gold standard of chlamydial serology (31) with specificity estimated at 83% to 89% and sensitivity at 79% to 86% (32, 33). Elementary bodies of pooled serovars BED (B-group), CJH (C-group), and GFK (intermediate group) of C. trachomatis (Washington Research Foundation, Seattle, WA) and Kajaani 6 strain of C. pneumoniae were used as antigens. FITC-conjugated antihuman IgG (Kallestad, Chaska, MO) was used as conjugate. The serum samples were analyzed at 2-fold dilutions for C. trachomatis and at 4-fold dilutions for C. pneumoniae. Titers of ≥16 were considered positive for C. trachomatis IgG antibodies and titres of ≥32 for IgG were considered positive for C. pneumoniae. The antibody determinations of each case and the individual controls were always done simultaneously in the same titration series in a blinded fashion. Positive control serum was included in every test series. All the sera were tested by one laboratory technician and fluorescence pattern was interpreted by one experienced reader (T.A.).

Statistical Methods. To describe the data, we presented the prevalences of the different chlamydial exposures among cases and controls. However, to maintain the matching of the case-control design in the analysis, the estimation of the effect of exposure was done by calculating the odds ratios (OR) and their 95% confidence intervals (95% CI) using conditional logistic regression analysis. First, to give an overall view (the OR) of the effect of C. trachomatis on prostate cancer risk, the variable describing the exposure, the IgG antibody titer level, was dichotomized using the above described limit values for positive exposure. Next, to explore the pattern of dose-response, the exposure variable was categorized at three levels (e.g., for C. trachomatis the titer levels of <16, 16, and ≥32 represented “no exposure”, “intermediate exposure”, and “elevated exposure” respectively. Similarly, titer levels for C. pneumoniae were classified to no exposure (<32), intermediate exposure (32-64), and elevated exposure (≥128). A consistent trend in the estimated ORs for these exposure levels is considered to strengthen the inference based on the overall OR.

Results

Of the 738 cases with prostate carcinoma, 514 were from Norway, 138 were from Finland, and 86 were from Sweden. At the time of serum sampling the mean age of the cases was 48.9 years (range 34-73 years) and the mean age at diagnosis was 62.9 years (range 44-79 years). The median time between withdrawal of serum and cancer diagnosis was 14 years (range 0-25 years; Table 1). In the total cohort, 66% of the serum samples had been taken before 1980, 19% between 1980 and 1984, and 15% after 1985. Of all cases, 493 (67%) were localized, 178 (24%) were nonlocalized, and in 67 (9%) the stage was unknown.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All cohorts, n = 738</th>
<th>Norway, n = 514</th>
<th>Finland, n = 138</th>
<th>Sweden, n = 86</th>
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<tr>
<td>Age at serum sampling</td>
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<td>&lt;45</td>
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<td>146 (28.4)</td>
<td>12 (8.7)</td>
<td>1 (1.2)</td>
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<td>45-50</td>
<td>366 (49.6)</td>
<td>319 (62.1)</td>
<td>38 (27.5)</td>
<td>9 (10.5)</td>
</tr>
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<td>51-60</td>
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<td>44 (8.6)</td>
<td>88 (63.8)</td>
<td>68 (79.1)</td>
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<tr>
<td>&gt;60</td>
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<td>5 (1.0)</td>
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<td>8 (9.3)</td>
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<td>Age at diagnosis</td>
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<tr>
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<td>1 (0.2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<td>45-50</td>
<td>20 (2.7)</td>
<td>16 (3.1)</td>
<td>2 (1.4)</td>
<td>2 (2.3)</td>
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<tr>
<td>51-60</td>
<td>189 (25.6)</td>
<td>124 (24.1)</td>
<td>47 (34.1)</td>
<td>18 (20.9)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>528 (71.5)</td>
<td>373 (72.6)</td>
<td>89 (64.5)</td>
<td>66 (76.7)</td>
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<td>Lag between serum sampling and diagnosis (years)</td>
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<td></td>
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</tr>
<tr>
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<td>1 (0.2)</td>
<td>1 (0.7)</td>
<td>11 (12.8)</td>
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<tr>
<td>1-4</td>
<td>79 (10.7)</td>
<td>16 (3.1)</td>
<td>11 (8.0)</td>
<td>52 (60.5)</td>
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<tr>
<td>5-9</td>
<td>102 (13.8)</td>
<td>49 (9.5)</td>
<td>30 (21.7)</td>
<td>23 (26.7)</td>
</tr>
<tr>
<td>10-14</td>
<td>181 (24.3)</td>
<td>85 (16.5)</td>
<td>96 (69.6)</td>
<td>0 (0.0)</td>
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<td>&gt;15</td>
<td>363 (49.2)</td>
<td>363 (70.6)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<td>Time of serum sampling</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Before 1980</td>
<td>487 (66.0)</td>
<td>487 (94.7)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>1980-1984</td>
<td>141 (19.1)</td>
<td>3 (0.6)</td>
<td>138 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>1985-1989</td>
<td>35 (4.5)</td>
<td>24 (4.7)</td>
<td>0 (0.0)</td>
<td>9 (10.5)</td>
</tr>
<tr>
<td>After 1989</td>
<td>77 (10.4)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>77 (89.5)</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localized</td>
<td>493 (66.8)</td>
<td>365 (71.0)</td>
<td>66 (47.8)</td>
<td>62 (72.1)</td>
</tr>
<tr>
<td>Nonlocalized</td>
<td>178 (24.1)</td>
<td>127 (24.7)</td>
<td>36 (26.1)</td>
<td>15 (17.4)</td>
</tr>
<tr>
<td>Not determined</td>
<td>67 (9.1)</td>
<td>22 (4.3)</td>
<td>36 (26.1)</td>
<td>9 (10.5)</td>
</tr>
</tbody>
</table>
The presence of serum antibodies against all serotype pools of C. trachomatis and against C. pneumoniae was higher among controls than among cases (Table 2). In BED—the commonest pool—the prevalence of C. trachomatis antibodies was 7% in cases and 10% in controls, and in pools combined the figures were 8% and 11%, respectively. C. pneumoniae antibodies were found in 48% of cases and in 52% of controls (Table 2). No differences were found in prevalences by age, lag time, or stage of the disease (data not shown).

The prevalences were further transformed into ORs. Serum antibodies to C. trachomatis were inversely associated with prostate cancer risk showing no major differences in the point estimates of ORs with regard to different C. trachomatis serotype groups (Table 3). In total cohort (Norway, Finland, and Sweden) the risk for prostate cancer was significantly lower in Norway (OR, 0.83; 95% CI, 0.67-1.02) than in Finland (OR, 0.71; 95% CI, 0.40-1.29) and somewhat, but not statistically significant (OR, 0.72; 95% CI, 0.46-1.14); and for those with localized cancer the OR was 0.67 (95% CI, 0.45-1.00) and for those with nonlocalized cancer the OR was 0.69 (95% CI, 0.37-1.29).

Discussion

In the present prospective case-control study, we show that C. trachomatis antibodies were not positively associated to the risk of prostate cancer, but their presence seemed to protect against cancer. The finding was the same in all three countries, Finland, Sweden and Norway, and the effect was even stronger for the higher titres of C. trachomatis antibodies. The same inverse relationship, although not so strong, was also seen between antibodies to C. pneumoniae and prostate cancer.

In the present study, we used the same microimmunofluorescence method, which we have successfully used in our earlier seroepidemiologic studies for the measurement of antibodies to C. trachomatis and C. pneumoniae (17, 18, 34). Antibodies are reasonably stable for several years in serum at –25°C (27). It has been shown that chlamydial IgG antibodies do not deteriorate even with very long storage time (35).

When stratified ORs were estimated by age at serum sampling or by lag time between sampling and diagnosis or by stage of the disease at diagnosis, the inverse association between C. trachomatis and prostate cancer risk remained persistently at the same level with OR of about 0.7. For those ≤50 years of age the OR was 0.71 (95% CI, 0.49-1.02) and for those >50 years of age the OR was 0.67 (95% CI, 0.37-1.22). Similarly for those with lag time ≤15 years the OR was 0.65 (95% CI, 0.42-1.00) and for those with lag time >15 years the OR was 0.72 (95% CI, 0.46-1.14); and for those with localized cancer the OR was 0.67 (95% CI, 0.45-1.00) and for those with nonlocalized cancer the OR was 0.69 (95% CI, 0.37-1.29).

Table 4. ORs for prostate cancer by increasing levels of IgG antibody titers to C. trachomatis and C. pneumoniae

<table>
<thead>
<tr>
<th>C. trachomatis</th>
<th>C. pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titer level</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>&lt;16</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>0.75 (0.53-1.06)</td>
</tr>
<tr>
<td>≥32</td>
<td>0.57 (0.34-0.96)</td>
</tr>
</tbody>
</table>

When stratified ORs were estimated by age at serum sampling or by lag time between sampling and diagnosis or by stage of the disease at diagnosis, the inverse association between C. trachomatis and prostate cancer risk remained persistently at the same level with OR of about 0.7. For those ≤50 years of age the OR was 0.71 (95% CI, 0.49-1.02) and for those >50 years of age the OR was 0.67 (95% CI, 0.37-1.22). Similarly for those with lag time ≤15 years the OR was 0.65 (95% CI, 0.42-1.00) and for those with lag time >15 years the OR was 0.72 (95% CI, 0.46-1.14); and for those with localized cancer the OR was 0.67 (95% CI, 0.45-1.00) and for those with nonlocalized cancer the OR was 0.69 (95% CI, 0.37-1.29).
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the Nordic countries (10, 36−39). *C. trachomatis* titer 1:16 was used in this study. We repeated the analysis with 1:8 as a cutoff. As expected, this resulted in estimates of higher prevalence and lower protective effect probably because of the unspecific background noise.

Our study population was well defined. The controls were matched for obvious confounders, which makes selection bias unlikely. Evidence based on longitudinal studies is stronger than that based on cross-sectional case-control studies. Serum banks and cancer registries are ideal for systematic evaluation of exposure to chlamydial infections and for identification of individuals who reach the end point. The sample size of the present study was large and thus the random fluctuation remains small. Furthermore, the higher antibody prevalence in controls compared with cases was found consistently in different countries and there was a dose-response gradient.

In an earlier Finnish study with another serum bank (10) no association (RR = 1.0) was found between *C. trachomatis* antibodies and risk of prostate cancer. The numbers were few, however, and based on the confidence interval (0.5−2.0) the result was also consistent with the 30% protective effect. We have indicated earlier that women with *C. trachomatis* antibodies were at increased risk of cervical squamous cell carcinoma but an inverse association was seen between *C. trachomatis* antibodies and cervical adenocarcinoma (17). Difference by histologic type of tumor was shown also between lung cancer and *C. pneumoniae* antibodies: the presence of antibodies was linked to the squamous cell carcinoma but not to adenocarcinoma (34). Prostate cancers are almost uniformly adenocarcinomas. Therefore, the present study is consistent with some of the previous ones, which, however, do not explain the finding.

A possibility is that the presence of *C. trachomatis* in adenoid cells of prostatic tissue does not induce the formation of serum antibodies. Recently, it has been suggested that lack of IgA antibodies to the Hsp60s of *C. trachomatis* predisposes to male infertility (40). However, the role of serology in the search of correlation between *C. trachomatis* infection and male infertility has been questioned (41−43). Prostate tissue secretes immunosuppressive proteosomes (44) and seminal fluid contains high levels of immunosuppressive prostaglandins (45), which may hamper the development of humoral antibody response even in cases in which *C. trachomatis* is present in the tissue. This could be studied by direct demonstration of the agent in prostatic tissue (46, 47) which was not available in the present study. Immunologic mechanisms might explain the lack of positive association but are not credible explanations of the inverse relationship observed.

The results of the present study further suggest similar association of both *C. trachomatis* and *C. pneumoniae* antibodies with the risk of prostatic cancer. Misclassification (e.g., due to cross reactivity) can account for such a relationship. However, more complex, and differential, misclassification is to be assumed for the inverse association between one or both of the infectious agents and risk of prostate cancer. It is also possible that if a person has a chlamydial infection in other parts of the body, the provoked inflammatory response of the host against these infections may indirectly help the host to fight against other microbial agents possibly inhibiting the prostatic cancerous process and by that way protect from prostatic cancer. Mycobacterial vaccines have been suggested to be useful in immunotherapy of prostate cancer (48, 49). Chlamydiae have been shown to inhibit apoptosis of the cells in which they are hiding, but induce the apoptosis of the other cells (e.g., T cells present in the neighborhood (50), and by this way may destroy important target cells for other microbial agents.

It is also possible that chlamydial antibodies are an indirect indicator of factors preventing exposure to risk factors of prostate cancer or reducing the risk of prostate cancer. Treatment of chlamydia or any disease sufficiently correlated with chlamydia infection is the most immediate option. There are examples on such a preventive action (51). We observed a similar inverse relationship between *C. pneumoniae* and risk of prostatic cancer, which in principle can be further evidence for such a mechanism. *C. trachomatis* is often symptomless in males and *C. pneumoniae* has a variety of symptoms, which makes it unlikely that men positive for these two infections were subjected to substantially higher doses of, say, antibiotics than the controls. The hypothesis of the treatment effect is to assume high correlation with a third condition (e.g., gonorrhea) systematically treated with relatively specific substances and high doses. Such a third exposure may also belong to sexual habits, such as ejaculation frequency (52), hormonal balance (53), or even more remote environmental factors (54). We showed in the present study that chlamydial antibodies are more common in controls than in cases of prostate cancer. We conclude that it is unlikely that *C. trachomatis* is increasing the risk of prostate cancer. Whether *C. trachomatis* infection prevents prostate cancer remains open. Competing explanations are related to an unidentified confounder, such as to the effects of treatment of chlamydia or to a related disease or to the immunologic response to a true etiologic agent or to the incapability of measuring the true exposure in prostatic tissue by serology.

References


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