Diagnostic Value of Serological and Molecular Methods for Screening and Detecting Chlamydia trachomatis Infection in Semen Samples of Infertile Men Referring to Royan Institute

Authors:

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INTRODUCTION

- Genital tract infection and inflammation have been associated to 8-35% of male infertility
- *Chlamydia trachomatis* is the most common etiological agent of sexually transmitted diseases after Herpes simplex Virus and Human papilloma Virus.
- CT is the most prevalent bacterial cause of sexually transmitted infections in the world
The reported incidence rates of genital chlamydial infections in the population are likely an underestimate because of the highly asymptomatic nature of the pathogen.

Approximately, 75% of infected women and 50% of infected men have asymptomatic urogenital infections, representing a huge population of untreated individuals who can transmit the organism.
Over 90 million chlamydial infections are detected annually worldwide and various studies have estimated that there are four to five million new cases of chlamydial infection each year only in the USA.
Figure 46-1 The life cycle of chlamydiae. The entire cycle takes approximately 48 to 72 hours.
DETECTION METHODS

- Cell Culture: McCoy or Buffalo green Monkey cells
- Direct Fluorescent Antibody Tests; DFA
- Enzyme Immunoassays
- Nucleic Acid Hybridization test
- Nucleic Acid Amplification tests
STUDY OBJECTIVES

➢ To determine the diagnostic value of serological and molecular methods for screening and detecting Chlamydia trachomatis infection in semen samples of infertile men presenting to the Royan Institute.

➢ Comparing serological and molecular results to determine an appropriate method for screening CT in infertile men.
MATERIALS & METHODS

- A cross sectional study, 465 infertile men (93 Asymptomatic & 372 Symptomatic)
- ELISA screening test for Anti-CT IgA in seminal plasma
- DNA extraction and Real-Time PCR for confirming the results
RESULTS & DISCUSSION
Infertile men
465

Symptomatic
372

ELISA OD<0.200
340

ELISA OD>0.200
32
(OD>0.400=7)

PCR Negative
28

PCR Positive
4
(ELISA OD>0.400)

Asymptomatic
93

ELISA OD>0.200
30
(OD>0.400=2)

PCR Positive
1
(ELISA OD>0.400)

PCR Negative
29
The results of ELISA test according to OD of samples

<table>
<thead>
<tr>
<th>Patient’s Classification</th>
<th>Number</th>
<th>Cut-off OD=0.200</th>
<th>Cut-off OD=0.400</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OD&gt;0.200</td>
<td>OD&lt;0.200</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>372(80)</td>
<td>32(52)</td>
<td>340(84)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>93(20)</td>
<td>30(48)</td>
<td>63(16)</td>
</tr>
<tr>
<td>Total</td>
<td>465</td>
<td>62</td>
<td>403</td>
</tr>
</tbody>
</table>

Data are shown as n (Percentage)
Frequency of CT infection in study groups resulted by means of PCR

<table>
<thead>
<tr>
<th>C. trachomatis</th>
<th>Symptomatic</th>
<th>Asymptomatic</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>4 (12.5%)</td>
<td>1 (3.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Negative</td>
<td>28 (87.5%)</td>
<td>29 (96.7%)</td>
<td>34 (100%)</td>
</tr>
</tbody>
</table>

Data are shown as n (Percentage)
The results of semen parameters in study groups

<table>
<thead>
<tr>
<th>Case groups</th>
<th>Symptomatic (n = 32)</th>
<th>Asymptomatic (n = 30)</th>
<th>Control (n = 34)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume (ml)</td>
<td>2.84 ± 1.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.12 ± 1.41&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.07 ± 1.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.002</td>
</tr>
<tr>
<td>Sperm concentration (x10&lt;sup&gt;6&lt;/sup&gt;/ml)</td>
<td>39.64 ± 30.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.57 ± 32.84&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>79.65 ± 33.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>28.21 ± 14.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.35 ± 11.02&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>57.99 ± 11.70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sperm morphology (%)</td>
<td>2.53 ± 2.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.93 ± 1.66&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.32 ± 1.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WBC in semen (x10&lt;sup&gt;6&lt;/sup&gt;/ml)</td>
<td>0 (0-1.7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 (0-0.8)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0 (0-0)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

WBC, white blood cell; Data are presented as Mean±SD or Median (min-max) when appropriate. Different letters indicate significantly different means (Tukey post-hoc test of the one-way ANOVA).
Male urogenital tract infections are discussed as one of the significant etiological factors for male's infertility worldwide. 

According to previous studies, the prevalence of CT genital infection in some developed countries ranges from 1% to 25%, considering sexual orientation, number of partners and socioeconomic status of patients.

Perhaps because of social conservatism in Iranians regarding free sexual attitudes, the prevalence of sexually transmitted diseases including CT genital infection may not be as high as in more-developed countries, but the results of several studies show that appropriate preventive strategies for CT should be considered in our country.
In our study, the prevalence of ELISA positive (OD > 0.400) was 1.9% (95% CI 1.0–3.7), and 4 out of 32 (12.5%) symptomatic patients and 1 out of 30 (3.3%) asymptomatic patients were PCR-positive.

The discrepancy in prevalence of chlamydia infection between current study and previous investigations may be caused by differences in culture, religious commitment such as avoiding sex outside marriage, different sample sizes and types, the time period of sampling, and diagnostic methods.
CONCLUSION

✅ Chlamydia trachomatis urogenital infection should be timely diagnosed and well treated, otherwise it will lead to pelvic inflammatory disease (PID) and the other types of urogenital sequela in patients.
Screening of infertile men who do not show any clinical symptoms look unavoidable and can be considered as a part of the program of sexually transmitted disease (STD) control.
The Anti-CT IgA ELISA test could be introduced as an appropriate tool for screening purpose in seminal plasma of infertile men.
REFERENCES


Thanks for your attention