Screenning of *Chlamydia trachomatis* Infection among Women Attending Outpatient Clinic of Infertility

Heloisa Lopes Lavorato¹, Natália Prearo Moço¹, Laura Fernandes Martin¹, Ana Gabriela Pontes Santos², Anaglória Pontes², Marli Teresinha Cassamassimo Duarte³, Márcia Guimarães da Silva¹*

¹Department of Pathology, Botucatu Medical School, São Paulo State University, UNESP, Botucatu, Brazil
²Department of Gynecology and Obstetrics, Botucatu Medical School, São Paulo State University, UNESP, Botucatu, Brazil
³Department of Nursing, Botucatu Medical School, São Paulo State University, UNESP, Botucatu, Brazil

Email: *mgsilva@fmb.unesp.br

Received 5 August 2015; accepted 15 September 2015; published 18 September 2015

Abstract

Objective: The goal of this study was to determine the prevalence of *C. trachomatis* in women diagnosed with infertility attending the Outpatient Clinic of Infertility from Botucatu Medical School, UNESP, Brazil. Patients and Methods: This molecular study enrolled a total of 112 women. Among these patients, 62 presented primary infertility while 50 presented secondary infertility. The criteria for eligibility included women who were: reproductive-aged; no prior report of seroconversion for HIV; no antibiotic or vaginal cream used in the preceding 30 days; and abstinence from sexual intercourse for 72 hours before the visit. The women were submitted to a gynecological examination and cervical samples were collected with an endocervical cytobrush for molecular analysis of *C. trachomatis*. Results: The prevalence of chlamydial infection was 8% with similar prevalence between primary (8.1%) and secondary (8.0%) infertility. Conclusion: Considering the asymptomatic nature of chlamydial infection and its association with tubal factor infertility, there is a pressing need to incorporate the screening of *C. trachomatis* infection as part of the routine investigation for infertility. The early diagnostic by screening can minimize complications and reduce Public Health costs with Assisted Reproductive Technology.

*Corresponding author.

Keywords
Chlamydial Infection, Infertility, Molecular Diagnosis

1. Introduction

Infertility is a worldwide health problem among couples with approximately 15% current global infertility rate, translating to one in 6 couples suffering from this condition. Infertility is defined as the inability of sexually active couples taking no contraceptives to achieve pregnancy within 1 year. Primary infertility is used to describe a couple that has never been able to conceive a pregnancy, while secondary infertility is used to describe the inability to become pregnant following the birth of one or more biological children [1].

According to the World Health Organization, the etiology of infertility is multifactorial, including female, male and combined male and female factors and in some cases without known etiology. A study carried out with 8500 infertility couples shows that 37% are due to female factor, 8% due to male factor, and 35% due to combined male and female factor infertility. The most common identifiable factors associated with female infertility are ovulatory disorders (25%) and tubal pathologies (22%), followed by endometriosis (15%), pelvic adhesions (11%), and hyperprolactinemia (7%) [2].

The tubal factor infertility (TFI) is often caused by previous episode of pelvic inflammatory disease (PID) which is a polymicrobial infection of the upper genital tract. The diagnosis of PID is based primarily on clinical evaluation and the principal symptoms are lower abdominal or pelvic pain and cervical motion or pelvic tenderness [3]. Tubal tissue damage occurs when acute PID progresses into a chronic form of the disease resulting in scarifications and adhesions in the fallopian tubes [4]-[6]. The relative risk for TFI after a single episode of PID is approximately 10% and this risk doubles after each recurrent episode [6].

Sexually transmitted infections (STI) are often considered the leading preventable cause of infertility worldwide because they are responsible for most cases of PID [7]-[9]. Chlamydial infection, caused by the bacterium Chlamydia trachomatis, is one of most frequently reported STI [2].

C. trachomatis, an obligate intracellular human pathogen, is a Gram-negative bacterium with a circular genome of 1042 kbp [10] [11]. It can appear as either spherical or ovoid shape [9]. Life cycle of C. trachomatis is composed by two distinct stages named elementary body (EB) and reticulates body (RB). The EB is the extracellular and infectious form while RB is the intracellular, noninfectious and metabolically active form [8] [12] [13].

According to the World Health Organization in 2008, the total number of new cases of C. trachomatis in adults was 105.7 million worldwide. The prevalence of C. trachomatis in women between 15 - 49 years in Americas was 7.6%, as well as 2.6% in Africa, 1.1% in South-East Asia and 3.9% in Europe [2]. Chlamydial infection is asymptomatic in the most cases and delay in its diagnostic may cause several harmful effects mainly in women [14]-[18].

Treatment of PID and TFI engender elevated financial and psychological costs. Screening programmes for chlamydial infection have been implemented in order to decrease these costs. The major aims of C. trachomatis screening are to reduce morbidity by early detection and treatment of lower genital tract infection as well as to decrease prevalence of this infection and consequently reduce their transmission [19]. Randomized controlled trial conducted in the USA demonstrated that selective C. trachomatis screening reduced the incidence of PID by 56% after 1 year of follow up [20]. In addition, for every 83 women screened, one case of PID is prevented [21]. Moreover, several studies suggest that screening of this infection becomes cost-effective at prevalence of 2% - 10% [22]-[25].

Several studies have been shown the association between chlamydial infection and TFI [26]-[30]. The prevalence of C. trachomatis in infertile women ranges from 3.9% to 32% worldwide [26]-[28] [31]-[39]. The discrepancy between these results could be attributed to differences in the sampling, methodology and type of biological sample [9] [40] [41].

The purpose of this study was to determine the prevalence of C. trachomatis in women diagnosed with primary and secondary infertility attending the Outpatient Clinic of Infertility from Botucatu Medical School, UNESP, Brazil.
2. Material and Methods

2.1. Study Population

This molecular study enrolled a total of 112 women attended the Outpatient Clinic of Infertility of Botucatu Medical School, São Paulo, Brazil. All women that received assistance in this outpatient between July 2008 and June 2009 were included. Among these patients, 62 presented primary infertility while 50 presented secondary infertility. The criteria for eligibility included women who were: reproductive aged; no prior report of seroconversion for HIV; no antibiotic or vaginal cream used in the preceding 30 days; and abstinence from sexual intercourse for 72 hours before the visit. (Sociodemographic and gynecology characterization of the study groups were performed by interview from all the women enrolled. Following the interview, the women were submitted to a gynecological examination.

The Human Research Ethics Committee of the institution approved the study and all patients provided written informed consent before enrollment.

2.2. Sample Collection

After inserting a non-lubricated speculum, cervical samples were collected with an endocervical cytobrush for molecular analysis of *C. trachomatis* by Polymerase Chain Reaction (PCR). Endocervical samples were inserted in 1 mL of Tris-EDTA-Tween solution that was stored at −20°C until analysis.

2.3. Detection of *C. trachomatis*

For the evaluation of *C. trachomatis* infection by PCR, DNA extraction was performed with a previous digestion with proteinase K. The presence and integrity of DNA samples was confirmed by the amplification of beta-globin constitutive gene using primers GH20 (GAA GAG CCA AGG ACA GGT AC) and PCO4 (CAA CTT CAT CGT TCA CC) which results in amplification of a 268 bp sequence. *C. trachomatis* was detected by the amplification of a 201 bp sequence by PCR, using Taq DNA Polimerase (Platinum, Invitrogen®) and primers CTP1 (TAG TAA CTG CCA CTT CAT CA) and CTP2 (TTC CCC TTG TAA TTC GTT GC) (Fig. 1B) [42]. Amplification parameters consisted in 39 cycles of 1 min at 95°C, 1 min at 55°C and 30 s at 72°C. In all reactions, negative and positive controls were run simultaneously, with respectively sterile water and CT DNA extracted from infected McCoy cells. Amplicon molecular weight was determined by comparing to a standard size marker, under UV transilumination.

2.4. Data Analysis and Statistics

Sociodemographic data and sexual history from patients included in this study were presented in percentage while gynecologic data was presented as percentage and median followed interquartile range. The prevalence of *Chlamydia trachomatis* in the primary and secondary infertility groups was compared using Qui-square test (Sigma Stat 3.1).

3. Results

The sociodemographic and gynecologic characteristics and sexual history of the women included in the study are showed in Table 1.

The median of patient age, menarche and first sexual intercourse was 28 (14 - 44), 12 (9 - 17) and 16 (11 - 38) years, respectively. The median of fertility duration was 4 (1 - 17) years.

The prevalence of chlamydial infection was 8% (9/112). Among infertile women with positive chlamydial infection, pelvic pain was reported by 22.2% (2/9) patients, while dyspareunia, and previous treatment for lower genital tract infection was reported by 44.4% (4/9), and 77.7% (7/9), respectively. There was no statistically significant difference in the chlamydial infection prevalence between primary (5/62) and secondary (4/50) infertility (p = 0.736). Regarding secondary infertility, 50% (2/4) of patients with chlamydial infection have presented previous miscarriage.

4. Discussion

*C. trachomatis* is one of the most common worldwide-distributed bacterial sexually transmitted infections. Ac-
Table 1. Sociodemographic and gynecologic characteristics and sexual history of the women included in the study.

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>83</td>
<td>74.1</td>
</tr>
<tr>
<td>Non-white</td>
<td>29</td>
<td>25.9</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>27</td>
<td>24.1</td>
</tr>
<tr>
<td>≥25</td>
<td>85</td>
<td>75.9</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>Married</td>
<td>110</td>
<td>98.2</td>
</tr>
<tr>
<td><strong>Population of city of residence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100,000</td>
<td>75</td>
<td>66.7</td>
</tr>
<tr>
<td>&gt;100,000</td>
<td>37</td>
<td>33.3</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>96</td>
<td>85.7</td>
</tr>
<tr>
<td>Smoker</td>
<td>16</td>
<td>14.3</td>
</tr>
<tr>
<td><strong>Employment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>71</td>
<td>63.4</td>
</tr>
<tr>
<td>No</td>
<td>41</td>
<td>36.6</td>
</tr>
<tr>
<td><strong>No. sexual partners</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>=1</td>
<td>48</td>
<td>42.9</td>
</tr>
<tr>
<td>2 - 3</td>
<td>43</td>
<td>38.3</td>
</tr>
<tr>
<td>≥4</td>
<td>21</td>
<td>18.8</td>
</tr>
<tr>
<td><strong>No. sexual intercourse/week</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3</td>
<td>36</td>
<td>32.1</td>
</tr>
<tr>
<td>&gt;3</td>
<td>76</td>
<td>67.9</td>
</tr>
<tr>
<td><strong>Menarch</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>98</td>
<td>87.5</td>
</tr>
<tr>
<td>≥15</td>
<td>14</td>
<td>12.5</td>
</tr>
<tr>
<td><strong>First sexual intercourse age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>24</td>
<td>21.4</td>
</tr>
<tr>
<td>15 - 20</td>
<td>71</td>
<td>63.4</td>
</tr>
<tr>
<td>≥20</td>
<td>17</td>
<td>15.2</td>
</tr>
</tbody>
</table>

cording to a study published by the World Health Organization in 2012, the number of new cases of chlamydial infection has been estimated globally to be 105.7 million in 2008. Although the high incidence of this infection, the real prevalence is difficult to estimate without a screening as the most cases are asymptomatic [16]-[18].

The prevalence of chlamydial infection in infertile women in this study was 8%. Similar prevalence was found in studies carried out in Oman (5.5%) [43], Central Saudi Arabia (8%) [26], Poland (8.7%) [44], Nigeria (9.6%) [35] and Brazil (10% and 10.9%) [39] [45]. However, higher prevalence was found by others studies carried out in Saudi Arabia (15%) [28], UK (16%) [36], India (28.1%) [46] and Iran (32%) [34]. Different pre-
valence around the world showed in these studies could be explained by the methodology employed, type of biological sample collected and population studied [9] [40] [41].

Our prevalence rate of *C. trachomatis* in endocervical samples from infertile women was obtained by conventional PCR. This high prevalence was expected due to characteristics of included women. Several studies reported that sexual behaviors such as early age of first intercourse and multiple partners are most common risk factors for this infection [47]-[50]. Alfarray et al. [26], using the same sample and the same methodology, found 8% of *C. trachomatis* in infertile women in Saudi Arabia. Similarly, in another study carried out in Brazil, Marques et al. [45] showed 10% of *C. trachomatis* in endocervical sample from women attended in Outpatient Clinic of Sterility using conventional PCR.

Traditionally chlamydial infection had been diagnosed by culture and rapid antigen detection methods. These methodologies had several limitations which included low sensitivity, long testing time and high cost [9] [51] [52]. Cell culture methods for *C. trachomatis* have a high specificity however the sensibility can range between 60% - 80%. Additionally, culture methods are difficult to be standardized and technically demanding [51] [52]. Rapid antigen-based testing such as direct fluorescent antibody (DFA) and enzyme immune assay (EIA) also have high specificity [53] [54] but DFA requires expertise in microscopic examination and interpretation of results [9] [51] [55] while the sensibility of EIA tests can vary between 60 - 96 per cent [54] [56]. The development of tests based on nucleic acid amplification technology (NAAT) led to the improvement of sensitivity for *C. trachomatis* diagnostics. The most widely employed NAAT is polymerase chain reaction (PCR) which is at least 20 - 30 per cent more sensitive and 100 per cent specific due to the ability to detect as little as a single copy of the gene [57]-[61].

Spread of *C. trachomatis* from endocervix to female upper genital tract causes PID. Tubal tissue damage resulting from previous episodes of PID leads to tubal blockage and consequently to TFI [7]-[9] [41]. According to Price et al., a single episode of *C. trachomatis* has a 16% of probability to result in PID [62]. Moreover, Wiesenfeld et al. [63] showed that women diagnosed with subclinical PID have a 40% reduced incidence of pregnancy.

A limitation of the present study is that it is restricted to 112 women attending the Outpatient Clinic of Infertility of Botucatu Medical School, São Paulo, Brazil and who rely on the public health system exclusively. Thus, these data on chlamydial infection prevalence might not be generalizable to all Brazilian women with infertility.

Given the asymptomatic nature of chlamydial infection and its association with tubal factor infertility, there is a pressing need to incorporate the screening of *C. trachomatis* infection as part of the routine investigation for infertility. The early diagnostic by screening can minimize complications and reduce Public Health costs with Assisted Reproductive Technology.

**References**


