Bilateral but not unilateral tubal obstruction is associated with positive chlamydia serology

Fabiana C Approbato1, Mário S Approbato1, Mônica C S Maia1, Yanna A R de Lima1, Maria A Barbosa2, Beatriz B do C Benetti3

1Brazil Human Reproduction Laboratory. Obstetric and Gynecology Dept. Federal University of Goias State, Brazil
2Nursing School. Federal University of Goias State, Brazil
3Nutrition School. Federal University of Goias State, Brazil

ABSTRACT
Objective: To assess the association between positive Chlamydia trachomatis (C. trachomatis) serology and unilateral or bilateral tubal obstruction.

Methods: This was a cross sectional study that evaluated the association of positive C. trachomatis serology (Immunofluorescence Indirect Serology, IIF or Enzyme Immune Essay, EIE), in two infertile groups: A. 243 patients (27 with unilateral obstruction and 216 without it). B. 247 patients (31 with bilateral obstruction and 216 without it). The exclusion criteria were tubal ligation (tubectomy) and tubal surgery. The statistical test (SPSS 17.0) was the Chi-Square with a p=5%. Tubal obstruction was diagnosed through hysterosalpingography (HSG).

Results: The mean age of the patients without obstruction was 33.6 years, SD 4.9. The mean age of the patients with unilateral obstruction was 33.7 years SD 4.9. The mean age of the patients with bilateral obstruction was 33.6 years, SD 4.9. There was no statistically significant difference between the age groups. In group A (unilateral obstruction versus serology) the Chi-Square was 0.02 (p=n.s.) and the Attributable Risk (AR) = 0.7%. In Group B (bilateral obstruction versus serology) the Chi-Square test was 9.87 (p<0.005) and the AR = 14.8%.

Conclusion: This study found a strong and statistically significant association between bilateral tubal obstruction and C. trachomatis positive serology. The power of the test was 86%. There was no association between unilateral obstruction and positive serology.

Keywords: tubal obstruction, Chlamydia trachomatis, C. trachomatis, tubal patency, infertility

INTRODUCTION
Chlamydia trachomatis infection is one of the most common sexually transmitted bacterial diseases, particularly in women (WHO, 2001). In 2009, a total of 1,244,180 Chlamydia infections were reported to the Centers for Disease Control (CDC) in 50 states and the District of Columbia, an increase of 2.8% when compared with 2008 (CDC, 2010). Even in developed countries the prevalence of C. trachomatis seems to be increasing. In Sweden, the incidence of Chlamydia infection increased from 171.7 to 406.2 cases per 100,000 between 1998 and 2009 (Sylvan & Christenson, 2008; Christenson & Sylvan, 2011).

The risk ratio of infection-induced reproductive diseases can be underestimated because up to 70%-80% of acute Chlamydia infections in women are asymptomatic or subclinical, and are not diagnosed or treated (Peipert, 2003). According to the World Health Organization, 10%-40% women with untreated or repeated infections develop symptomatic pelvic inflammatory disease (WHO, 2016), which results in scarring and fibrosis of the Fallopian tubes (Hillis et al., 1997) and can lead to ectopic pregnancy (Chow et al., 1990). Moreover, 30%-40% of cases of female infertility are caused by postinfectious tubal damage resulting in hydrosalpinx (Brunham & Rey-Ladino, 2005; WHO, 2016). Chlamydia infection treatment does not always prevent progressive tubal damage (Brunham & Rey-Ladino, 2005).

C. trachomatis infection has been on the rise worldwide and frequently causes tubal damage, often irreparable, of difficult management, limiting the reproductive capacity of women (WHO, 2001). Due to its serious consequences, C. trachomatis antibody testing is part of the infertility work-up suggested by the Dutch Society of Obstetrics and Gynecology (Dobekousen et al., 1994).

The prevalence of tubal pathology in female infertility varies depending on the author. Some published that it is estimated that 30% of infertile females have tubal pathology (Evers, 2002; Brandes et al., 2010). Dobekousen et al. (1994) found a total of 14% of tubal factor in female infertility. Between 10 and 15% of all women seeking In Vitro Fertilization in the United Kingdom are due to tubal infertility (HPFA, 2016).

Several hypotheses about tubal implantation have been proposed, including inhibition of ciliary beating and muscle contraction, stimulation of tubal secretion, and early embryotubal cell interaction (Shah et al., 2005). It has been speculated that an antibody response to the Chlamydia 60-kDa heat shocks the protein (hsp-60) and causes a tubal inflammatory response, leading to tubal blockage or a predisposition to tubal implantation (van Mourik et al., 2009; Daponte et al., 2012). There is evidence that the steroid hormones 17β-estradiol (E2) and progesterone (P4) increase susceptibility to Chlamydia infection and modulate inflammation in epithelial cells (Amirshahi et al., 2011; Hall et al., 2011).

The tubal secretory function is incompletely characterized, but epithelial cell secretions are known to affect gamete fertilization and early human embryo development. The makeup and volume of Fallopian tube fluid depend on physiological and pathophysiological conditions (Avilés et al., 2010). One consequence of tubal infection in mice is hydrosalpinx, as defined by tubal dilatation and abnormal fluid buildup (Shah et al., 2005). Hydrosalpinx has adverse effects on ongoing pregnancies and female fertility, perhaps by reducing endometrial receptivity (Cakmak & Taylor, 2011). Since successful intrauterine implantation requires a sustainable microenvironment (Jabour et al., 2006), an important question is how Chlamydia-induced
hydrosalpinx formation changes the local microenvironment and consequently triggers tubal implantation in women.

We did not find publications investigating whether or not unilateral tubal obstructions are associated with seropositivity for C. trachomatis serology. It was the goal of this paper to evaluate if seropositivity also relates to unilateral tubal obstruction.

MATERIALS AND METHODS

This was a cross-sectional study that evaluated the association of positive Chlamydia serology (Immunofluorescence Indirect Serology, IFI or Enzyme Immune Essay, EIE), in two infertile groups: Group A: 243 patients (27 with unilateral and 216 without tubal obstruction). Group B: 247 patients (31 with bilateral and 216 without tubal obstruction). The exclusion criteria were tubal ligation (tubectomy) and tubal surgery. The statistics assessment package used was the SPSS 17.0. The Chi-Square test yielded \( p = 5\% \). Tubal obstruction diagnosed was made by hysterosalpingography, which is a test part of the work-up of infertile couples, a minimally invasive method of evaluating tubal patency and is performed as the first line approach for assessing tubal pathology (Foroozanfard & Sadat, 2013).

RESULTS

The mean age of the patients without obstruction was 33.6 years, SD 4.9. The mean age of the patients with unilateral obstruction was 33.7 years, SD 4.9. The mean age of the patients with bilateral obstruction was 33.6 years, SD 4.9. There is no statistical difference between age groups. In group A (unilateral obstruction versus serology) the Chi-Square was 0.02 (\( p = \text{n.s.} \)), and the Attributable Risk (AR)=0.7\% (Figure 1). In Group B (bilateral obstruction versus serology) the Chi-Square test was 9.87 \( (p<0.005) \), with AR=14.8\% (Figure 2).

DISCUSSION

HSG is used worldwide to evaluate tubal patency. It is a simple method for assessing female sterility, it is a less expensive and elementary method to evaluate tubal pathologies, and can identify some congenital uterine anomalies. The advantage of laparoscopy is that it can identify some other pelvic abnormalities which may be the cause of infertility that cannot be detected by HSG, such as endometriosis, adhesions and tuberculosis. However, one limitation of HSG is that the interpretation of the images depends on the experience and skill of the radiologists involved (Foroozanfard & Sadat, 2013).

In a research, HSG was compared with laparoscopy, and the results showed that sensitivity was 65\% for tubal patency, but it increases the achievement of spontaneous pregnancy by three fold (Torre et al., 2010). Other authors in a cohort study investigated eighty-two infertile cases to compare tuboperitoneal factors by HSG and laparoscopy, and the results showed that pathological findings were seen in 45.1\% by HSG and 65.85\% by laparoscopy. The sensitivity and specificity of HSG were 63\% and 89.3\%, respectively, and the positive predictive value was 92\%, with a 55\% negative predictive value, and an accuracy ratio was 72\% (Sakar et al., 2008).

In a series of 360 infertile women, an initial hysterosalpingography study suggested an incidence of unilateral proximal tubal obstruction in 18, and bilateral obstruction in 22 women. When the HSG was repeated one month later, the unilateral obstruction persisted in 12 (3.3\%) and the bilateral obstruction in 9 (1.1\%) women (Dessole et al., 2000).

In the year 2018, the prevalence of tubal obstruction in a sample of 292 infertile patients seen at the Humana Laboratory of the Federal University of Goiás State had the following distribution: Uni or bilateral obstruction in 21.9\%, bilateral in 10.9\% and unilateral in 9.9\%. Serology (Immunofluorescence Indirect Serology, IIF or Enzyme Immune Assay, EIA) was positive for C. trachomatis in 27.1\%. PCR was positive in 0.94\% of patients. Steiner et al. (2015) published a study using a newer anti-Ct (anti Chlamydia trachomatis) assay developed by Geisler et al. (2012) that is, an elementary body-based ELISA that has been shown to have higher sensitivity and specificity than prior assays. Steiner et al. (2015) found that 19\% of the women in their sample were seropositive for anti-C. trachomatis IgG3. Approbato (2012) found in a Master of Science (MS) graduate
paper that 0.83% (120 patients) were PCR positive for *C. trachomatis*. In this same publication, this author found 36.5% of seropositivity in IIF or EIA.

Even in the presence of tubal patency, anti-*C. trachomatis* IgG3 seropositivity is associated with a lower likelihood of pregnancy and increased pregnancy complications. Steinier et al. (2015) found that positive anti-*C. trachomatis* diagnosis, using the new IgG3 test, women have as high as 3 times the risk of having an ectopic pregnancy. These same authors found that Anti-*C. trachomatis* IgG3 seropositive women were significantly less likely to conceive (risk ratio [RR] 0.65, 95% confidence interval [CI] 0.52-0.83) or to have a live birth (RR 0.59, 95% CI 0.43-0.80). To date, the specificity of the anti-Ct assay due to cross-reactivity with other *Chlamydia* strains has limited its clinical utility as a predictive test. Standard methodologies used to assess Fallopian tubes, assess the patency but not function (Feinberg, 2015). The utility of the improved detection method for anti-Ct antibody should be met with caution, because tubal patency does not imply tubal function. Seropositive patients should not be driven immediately for IVF treatments. But, it may be reasonable, in the setting of positive anti-Ct antibodies, to limit the number of ovulation induction cycles and pursue IVF if not pregnant after three cycles (Feinberg, 2015).

Titration of anti-*C. trachomatis* antibodies has been used to track tubal obstruction and pathologies. Rodgers et al. (2011) published that infertile women with laparoscopically identified tubal pathologies developed significantly higher titers of anti-*C. trachomatis* antibodies. Many publications (Mucish & Behrman, 1983; Serafini & Batzofin, 1989; Healy et al., 1994) described a prevalence of tubal obstruction and other pelvic diseases from 25% to 35% in patients with infertility, mainly by *C. trachomatis*. Nevertheless, there's no evaluation if these tubal obstructions were uni or bilateral. This paper is one of the first to show that unilateral obstruction is not related to seropositivity for *C. trachomatis*. It may be that in these cases, tubal spasms or false positivity are the probable causes. It seems that when there is an infection by this sexually transmitted disease, it affects both female tubes simultaneously. Other studies should be carried out to confirm these finds.

**CONCLUSION**

This study found a strong and statistically significant association between bilateral tubal obstruction and *Chlamydia* positive serology. The power of the test was 86%. There was not an association between unilateral obstruction and positive serology. This is one of the first published studies to show this.

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**CONFLICTS OF INTEREST**

The authors have no potential conflicts of interest, whether political, economic, of resources for research execution or intellectual property to declare.

**Corresponding Author:**
Fabiana C Approbato
Human Reproduction Laboratory/LabRep HC/GO Obstetric and Gynecology Dept.
Federal University of Goias State, Goias - Brazil.
E-mail: fabianapprobato@gmail.com

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