

## Brief Original Article

# Levels of different cytokines in women and men with asymptomatic genital infection caused by *Chlamydia*

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### Abstract

**Introduction:** Immune response to genital *Chlamydia trachomatis* infection is involved in both immunity and pathology. The cytokine profile during infection has been implicated in the disease outcome, either resolution or severe sequelae.

**Methodology:** In total, 3900 patients were analyzed for presence of genital infections caused by *Chlamydia* using molecular assays. Interleukins (IL) IL-10, IL-17, IL-6, IL-2 and chemokine IP-10 were estimated by ELISA in urine, cervical swabs and semen samples. Statistical analysis was performed using the T student test.

**Results:** A total of 47 out of 3900 samples (1.2%) were found to be positive for *Chlamydia trachomatis* based on the Real Time (RT) PCR results. Statistical analysis revealed that the differences between *Chlamydia trachomatis* positive and negative samples regarding levels of cytokines were not significant.

**Conclusions:** Our results demonstrated that no significant difference in cytokine concentrations exists in *Chlamydia trachomatis* infected patients when compared to healthy controls. In further study, we aim to test on a greater number of positive samples a greater number of cytokines involved in the immune response to *Chlamydia trachomatis* infections.

**Key words:** infection; *Chlamydia*; interleukin-10; interleukin-17; cytokine.

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### Introduction

*Chlamydia trachomatis* is a gram-negative bacterium living as an obligate intracellular parasite within the host cell [1]. *C. trachomatis* usually causes asymptomatic genital tract infections in both men and women, and the high prevalence of undiagnosed infected individuals provides a reservoir for spreading the infection to men and women through sexual contact [2]. The detrimental effects of *C. trachomatis* on female reproduction are more serious than the *Chlamydia* infections in men [3]. In women, this bacterium causes several diseases including cervicitis, endometritis, urethritis, acute and chronic pelvic inflammatory disease, infertility, and ectopic pregnancy [4]. In men, *C. trachomatis* may cause different urogenital infections, ranging from lower to upper genital tract diseases including urethritis, prostatitis, and epididymitis [5].

The role of *C. trachomatis* infection in the secretion of a number of cytokines from epithelial cells has been established by in vitro studies performed on various cell lines including ectocervical and endocervical cells, human urethral epithelial cells, and immortalized

normal adult male prostate epithelial cells [6-8]. A number of immunologic factors, including immunoglobulins, growth factors cytokines, and chemokines, have been detected in human semen and increased levels of these factors in semen from men with genital infections suggests their role in immune defense of the male genital tract [9-11]. Interleukin (IL)-10 and IL-17 are among the cytokines that have been more or less studied in genital tract infections including the ones caused by *C. trachomatis*. IL-10, also known as human cytokine synthesis inhibitory factor, is an anti-inflammatory cytokine that is an important regulator of several aspects of immune responses [12]. IL-17 is another pro-inflammatory cytokine which is secreted by activated Th17 lymphocytes. IL-17 has been associated with allergic inflammation and autoimmunity [13] and its role in immunity at the epithelial mucosal surfaces [14] and contribution to protection against intracellular bacteria has been documented [15].

In view of the above considerations, the present study was conducted to investigate the presence of IL-10, IL-17, IL-6, IL-10 and IL-2 in clinical samples of

men and woman who were PCR-positive for *Chlamydia* infection.

## Methodology

### Study population

Urine, cervical swabs and semen samples were collected between January 2013 and December 2015 from 3900 subjects. All samples were collected at the Unit of Microbiology of Hospital Agency of Sassari, a laboratory that provides microbiology services, including molecular testing for *C. trachomatis*, to hospital and outpatient locations.

Laboratory testing of *C. trachomatis* was requested for suspected of cervical, urethral or prostatitis infection or following infertility investigations.

### Molecular diagnosis

Laboratory diagnosis of *C. trachomatis* was performed using the Bio-Rad Dx CT/NG/MG assay (Bio-Rad, California, Hercules, USA) and was performed at the Unit of Microbiology of Hospital Agency of Sassari. Each sample (1 mL) was centrifuged for 10 minutes at 14000 rpm and a volume of 410 µL of the Bio-Rad lysis buffer containing internal control was added to the pellet. After vortexing, the samples were heated to 95 °C for 10 minutes and centrifuged for 2 minutes at 14000 rpm. Five µL of the supernatant was used for amplification with 20 µL mastermix of the Bio-Rad Dx CT/NG/MG assay in a Dx Real-Time System thermocycler (Bio-Rad, California, Hercules, USA) according to the manufacturer's instructions. The samples were automatically reported as positive or negative by the system's software.

### Cytokine measurement

Cytokines levels were detected in *C. trachomatis* positive or negative samples using ELISA technique (Sigma Aldrich-Merck, Dermastadt, Germany). Assays were performed according to the manufacturer's guidelines.

### Statistical analysis

Statistical analysis was performed using Graph Pad scientific calculator (Graph Pad Software, La Jolla, CA, USA). Quantitative data were compared using the Test t-student. A p value < 0.05 was considered statistically significant.

## Results

### Sociodemographic data

Among 3900 subjects 3276 (84%) were women and 624 (16%) were men. The mean age for women was 27

years and for men was 40 years. Ninety-eight percent of the subjects were Italians, 1.2% came from Eastern Europe and 0.8% were Africans. Most of the subjects were asymptomatic (65%). Male that were symptomatic reported needing to pass urine more often than usual; pain/burning when urinating; while symptomatic female reported itching; discharge; pain/burning when urinating; pelvic abdominal pain.

Six percent of women had fertility disorders and in whom the involvement of ovarian and tubal factors were the most frequent cause of infertility.

### Presence of *C. trachomatis* in genitourinary tract of study patients

A total of 47 from 3900 samples (1.2%) were found to be positive for *C. trachomatis* based on the Real Time PCR results. In women *Chlamydia* infections were identified in 28 cervical swabs (60% of the positive molecular results) while in men in 4 urine (8%) and in 15 semen samples (32%). All positive subjects for *C. trachomatis* were symptomatic: twenty (71%) women presented discharge, six (21%) pelvic abdominal pain and two (8%) had tubal infertility. All fifteen (100%) positive men for *C. trachomatis* had pain/burning when urinating.

### Comparison of interleukin level between *C. trachomatis* positive and negative patients.

All *C. trachomatis* positive samples and 36 negative samples: 3 urine (8%), 20 cervical swabs (55%) and 14 semen (37%) samples were analyzed in the study. Among the positive subjects for *C. trachomatis* 27 (57%) were Africans and 20 Italians (43%), while among the negative subjects 20 (55%) were Africans and 16 Italians (45%). The mean concentrations of IL-10 in PCR-positive samples were 3.7 pg/mL and in the negative samples were 1.9 pg/mL. The levels of IL-17 in the *C. trachomatis* positive group were 4.85 pg/mL and in the negative group were 4.65 pg/mL. The mean concentrations of IL-6 in subjects infected with *C. trachomatis* were 4.85 pg/mL and in the negative group were 4 pg/mL. IP-10 levels in positive *C. trachomatis* group were 4.75 pg/mL and in the negative group were 4 pg/mL. The levels of IL-2 in the *C. trachomatis* positive group were 3.28 pg/mL and in the negative group were 2.52 pg/mL. In two samples of cervical swabs of women with tubal infertility, we have detected high values of IL-10, IL-6 and IL-2 equal to 90 pg/mL and 80 pg/mL, 100 pg/mL and 92.5 pg/mL, 80 pg/mL and 70 pg/mL respectively.

### Statistical analysis

Statistical analysis revealed that the differences between evaluated groups regarding levels of IL-10, IL-17, IL-6, IP-10 and IL-2 were not significant ( $p = 0.52$ ,  $p = 0.71$ ,  $p = 0.572$ ,  $p = 0.232$ ,  $p = 0.758$  respectively).

### Discussion

Mucosal immune response to *C. trachomatis* infection of the female or men genital tract is not understood completely. In the present study, the relationship between levels of IL-10, IL-17, IL-6, IP-10, IL-2 in clinical samples and *C. trachomatis* infection was investigated. Our results demonstrated that no significant difference in cytokine concentrations exists in *C. trachomatis*-infected patients when compared to healthy controls. However, a strong production of IL-10, IL-6 and IL-2 was detected in two women with tubal infertility. In various studies, different levels of cytokines including IL-10 and IL-17 in male and female genital secretions contaminated with sexually transmitted infections, particularly, *C. trachomatis* infection, or of patients with infertility, have been reported. IL-10 is known to selectively suppress the production of different inflammatory cytokines, whose effects are needed to eradicate *C. trachomatis* infection [16]. Higher IL-10 levels were seen in infertile woman [17] and other studies found this cytokine to be associated with susceptibility and typical pathological changes caused by the genital *Chlamydia* infection such as granuloma formation and fibrosis [18]. Higher IL-10 levels in woman may be associated with tubal pathology and may be responsible for their failure to eradicate genital *Chlamydia* infection and the associated pathology. The relationship between IL-17 and immunity at the epithelial mucosal surfaces, protection against intracellular bacteria, male and female genital tracts, and infertility, was investigated in a few studies. Hakimi *et al.* showed that higher seminal levels of IL-10 and IL-17 were observed in patients with PCR-positive for *C. trachomatis* [19]. The present results are different from those of Hakimi *et al.*, while the reasons for this discrepancy are unknown but it might be speculated that differences in study populations or stage of infection at which the samples were taken may be the cause. Previous studies have found that progesterone suppresses Th17 response [20]. The low concentration of IL-17 that we found in women infected with *C. trachomatis* could be due to the use of progestin-only injectable hormone contraceptive.

Our study has several limitations: the small number of *C. trachomatis* positive samples analyzed and few cytokines measurement was performed. In further study

we aim to increase our series and test a greater number of cytokines involved in the immune response to *C. trachomatis* infection.

### Conclusion

Because no significant difference in cytokine concentrations exists in *Chlamydia trachomatis*-infected patients comparing to healthy controls, we cannot make any inference from this study regarding the diagnostic value. However the strong production of IL-10, IL-6 and IL-2 in women with tubal infertility suggests a possible value of measuring these cytokines in cervical swabs for diagnosis of genital infection and encourages us to carry out further investigations in this field.

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