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NGUYEN HOA

STUDY ON CLINICAL, PARACLINICAL CHARACTERISTICS AND GENOTYPE OF *Chlamydia trachomatis IN INFERTILE WOMEN AT NATIONAL HOSPITAL OF OBSTETRIC AND GYNECTOOGY* (2020-2021)

MEDICINE DOCTORAL THESIS

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Supervisors:

1. **Asso.Prof. Vu Van Du**
2. **Asso.Prof. Do Ngoc Anh**

Reviewer 1: Asso.Prof. Hoang Vu Hung

Reviewer 2: Asso.Prof. Doan Huy Hau

Reviewer 3: Asso.Prof. Le Tran Anh

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Thesis is always available in:

- Vietnam National Library
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INTRODUCTION

Chlamydia trachomatis (CT) is an obligate intracellular, gram-negative parasitic bacterium. This bacteria causing inflamatory diseases in the urinary tract. Besides it can also cause trachoma and pneumonia in children and immunocompromised adults. In women, CT infections of genital tract often have no symptoms or mild and nonspecific symptoms, therefore diagnosis and treatment are often overlooked. For woman with clinical manifestations, the common symptom is cervicitis - the cause of increased vaginal discharge, lower abdominal pain, vaginal bleeding and dysuria. This bacteria can also cause pelvic inflammation, salpingitis, fallopian tube blockage leading to infertility, ectopic pregnancy. This occurs in 10 -15% of women with CT.

Based on the structure of the cell membrane antigen encoded by the ompA gene, CT is classified into 19 genotypes including A, B/Ba, C, D/Da, E, F, G/Ga, H, I /Ia, J, K, L1, L2, L2a and L3. In which, Genotypes A-C are more common in trachoma, D-K are dominant in genitourinary infections and L1-L3 are dominant in lymphogranuloma venereum (LGV). The frequency of genotypes varies according to gender, race, sexual behavior and geographic region.

In Vietnam, diagnosis of CT infection in humans is not performed regularly and there are few studies analyze the CT genotype, especially in infertile women. Therefore, we carried out this study with the two following objectives:

1. To describle some clinical and paraclinical characteristics and some factors related to C. trachomatis infection in infertile women coming for exmination and treatment at NHOG (2020-2021).

2. To determine the genotype of C. trachomatis isolated from the participants.

SCIENTIFICITY, NEWNESS AND PRACTICALITY OF THE THESIS

1. THE NEWNESS

- This study is one of the few systematic study investigating on CT infection and related factors in infertile women in Vietnam.

- This is one of the new studies in Vietnam determining the genotypes of CT isolated from infertile patients.

- This study has identified 9 different CT genotypes in infertile patients: B/Ba, D/Da, E, F, G/Ga, H, I/Ia, J, and K. In which, Genotypes E, Da/D and F are the three genotypes with the highest proportion.

2. SCIENTIFICITY

- This study simultaneously used techniques and methods which are both classic and modern.

- To determine the rate of CT infection in infertile patients, the study used the cobas® CT/NG biological kit based on realtime PCR principles applied to Roche's Cobas® 4800 system. This is an IVD-issued biological kit for clinical diagnosis with high sensitivity and specificity.

- Genotypes were determined by sequencing and comparing the ompA gene sequence with the gene bank. The ompA gene sequences of the genotypes are registered and assigned codes in the gene bank.

- The project has identified 9 different genotypes of CT, including B/Ba, D/Da, E, F, G/Ga, H, I/Ia, J, and K. This result complements the data of CT genotype in Vietnam.

3. PRACTICALITY

- The results obtained from the study will become a reference for scientific research and teaching, and provide the knowlegdes for further research on CT infection in humans in Vietnam

- The study provides epidemiological data on CT infection in infertility women. The results obtained serve as a basis for effective prevention and treatment of diseases caused by CT.

STRUCTURE OF THE THESIS

The thesis consists of 132 pages, including: The introduction (2 pages); The literature review (31 pages); The participants and methods (28 pages); The results (34 pages); The discussion (32 pages); The conclusion (2 pages); The recommendation (1 page); 36 tables, 18 charts and 154 references.

Chapter 1. THE LITERATURE REVIEW

1.1. Biological characteristics of *Chlamydia trachomatis*

Chlamydia trachomatis (CT) is a obligate intracellular, gram-negative parasitic bacterium, belonging to the order Chlamydiales, family Chlamydiaceae. This is the most common sexually transmitted disease agent worldwide. Chlamydia's life cycle goes through two distinct forms: the elementary body (EB) and reticulate body (RB). The EB form is the infectious form, the RB form is the reproductive form of the bacteria. Chlamydia has poor tolerance, they are easily destroyed by heat, ultraviolet rays and common antiseptics. Therefore, this bacteria must be stored at subzero temperatures (-50 ^{0}C to -70 ^{0}C). Chlamydia is also sensitive to many groups of antibiotics such as penicillin, cephalosporin, tetracycline, erythromycin, sulfonamides, etc.

1.2. Genitourinary infections caused by C. trachomatis

CT can cause many different clinical conditions such as urethritis, cervicitis and LGV, trachoma, pneumonia in children and immunocompromised adults. However, genitourinary infections are the most common.

Characteristics of genitourinary infections caused by C. trachomatis

CT infection of genital tract often manifests subtle or asymptomatic (70%). A few cases of CT infection may experience painful urination, dysuria, increased vaginal discharge, or adnexitis. CT can also cause pelvic inflammatory disease, ectopic pregnancy and fertility; Pregnant women infected with CT can have miscarriages, premature births, stillbirths, etc.

Situation of C. trachomatis infection of genital tract

CT is the most common sexually transmitted pathogen worldwide. CT infection tends to increase. According to the World Health Organization (WHO), everyday there is about 1 million people infected with CT and 9.8% of childbearing – age women are infected with CT. By 2020, there were 129 million cases infeccted CT in the world and the figure is increasing. The rate of genital tract CT infection is highly variable, usually less than 5% in the community, but more than 10% in high-risk populations.

In Vietnam, the rate of women with genital tract CT infection is variable: The results of Nguyen Duy Anh (2022) was 9.62%, Pham My Hoai and et al (2022) 3.3%, Nguyen and et al

(2019) 6.0%, Nguyen Thi Nhu and et al (2013) 8.38%, Pham Van Duc and et al (2009) 9.1%, Tran Dinh Vinh and et al (2020) 15.6%, Le Hong Cam and et al (2001) 18.07%, Ho Thi My Chau and et al (2018) 26%, etc.

Factors related with genital C. trachomatis infection

CT infection often occurs in under 25 – year women, who have sex early, have sex with many partners, have STIs, and have a history of vaginitis and cervicitis.

Diagnosis of C. trachomatis infection of the genitourinary tract

Diagnosis of CT infection is based on epidemiological, clinical and paraclinical factors.

- Regarding epidemiology: the patient has a history of exposure to CT such as having sex or a partner with signs and symptoms of the disease.

- Regarding clinical: there are some symptoms such as cervicitis, endometritis, pelvic inflammation, urethritis, proctitis, etc.

- Regarding paraclinical: currently, immunology or molecular biology tests are often used to diagnose CT infection. The molecular biology method is approved by the Ministry of Health as the gold standard in diagnosing CT infection in Decision No. 5169/QD-BYT dated November 9, 2021.

It is necessary to differentiate infection from CT of urinary tract - genital tract infections caused by N. gonorrhoeae, trichomonas vaginalis, mycoplasma genitalium and other non-sexually transmitted agents.

1.3. Techniques for detecting C. trachomatis infection

CT can be detected by using microbiology, immunology or molecular biology methods. Among them, the nucleic acid amplification technique is recommended by the CDC of United State to test for diagnosis of CT infection due to its highest sensitivity and specificity. Immunology test is also applied in diagnosis. However, this technique has lower sensitivity and specificity than nucleic acid amplification technique.

1.4. Genotypes of C. trachomatis

Currently, 19 genotypes of CT including A, B/Ba, C, D/Da, E, F, G/Ga, H, I/Ia, J, K, L1, L2, L2a and L3 have been identified. These genotypes are all related to changes in the ompA gene of CT. Genotypes A-C are the cause of trachoma, D-K causes genitourinary infections, L1-L3 causes LGV. The prevalace of CT genotypes in the genital tract varies according to patients and geographical areas. In developed countries, genotypes D, E, and F are the most common causes of cervical, vaginal, and urethral CT infections. D, E, F are also the 3 dominant genotypes in infertile women. In Vietnam, data on CT genotypes is very limited. Research by Pham Van Bang and colleagues (2011) on 85 patients infected with genital tract CT with symptoms of genital inflammation showed that the three genotypes with the highest proportion were D (29.4%), F (27, 1%) and E (23.5%). Genotypes G, H, J, K have rates from 3.5% to 7.1%.

1.5. Infertility and relationship with CT infection

Infertility

According to WHO, Infertility is a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse.

Infertility can be divided into male infertility, female infertile or unknown cause. Or according to the status of pregnancy, it is classified into primary infertility and secondary infertility.

It is estimated that there are about 48.5 to 72.4 million couples with infertility problems worldwide. In childbearing – age people (15-49), the rate of infertility is about 10%-15%. In developed countries, the figure ranges from 3.5% to 16.7% and the figure for low-income countries ranges from 6.9% to 9.3%.

In Vietnam, the rate of infertility of young couples is about 7.7%. Of which, primary infertility is 3.9% and secondary infertility is 3.8%.

Association between CT infection and infertility

In men, CT can interfere sperm transport, potentially even causing changes in spermatogenesis, affecting sperm quality and quantity. In women, pelvic infection is one of the most important causes of infertility, especially infertility causing by fallopian tube. CT can move upstream, affecting the uterus, fallopian tube and ovaries, causing serious complications such as adhesion of the fallopian, accumulation of mucus in the fallopian, or causing adhesions and fluid retention, hindering the fertilization.

Chapter 2. THE PARTICIPANTS AND METHODS

2.1. The Participant, period, location and methods for the first objective: to describe some clinical and paraclinical characteristics and some factors related to C.trachomatis infection in infertile women coming for examination and treatment at the NHOG (2020-2021)

2.1.1. The participants

Infertile women aged from 19 to 52 years comming for examination and treatment at the NHOG from 2020 to 2021.

- Inclusion criteria: woman who came for examination and treatment for infertility at the NHOG during the research period; Patients volunteer to participate in the study and agree to provide information.

- Exclusion criteria: Women with neuropsychological abnormalities or acute internal or surgical diseases or HIV/AIDS infection; during menstruation, menorrhagia, bleeding; Place medication or douche within one week before the study period.

2.1.2. Research location: National Hospital Of Obstetric And Gynectoogy

***2.1.3.The period*:** 1/2020 to 12/2021

2.1.4. Study design and sample size

2.1.4.1. Research design: Analytical cross-sectional descriptive study.

2.1.4.2. Sample size and sampling method

- Sample size:

119 infertile women ages from 19 to 52 years old who infected with CT were characterized clinically and paraclinically. The number of infertile women is calculated according to the formula:

$$n = Z^2_{1-\alpha/2} \frac{p\,(1-p)}{d^2}$$

Particular:

+ n: The number of infertile women.

+ $Z^2_{1-\alpha/2}$: Value identified by statistical significance, $Z^2_{1-\alpha/2}$= 1,96 iif p-value is 5%

+ p: The incidence of a common symptom, refering to the incidence of abnormal vaginal discharge with p = 0.885 (p = 88.5%) in women infected with CT at Da Nang Obstetric and Pediatric Hospital in the study of Tran Dinh Vinh and et al (2020).

+ d: Absolutely acceptable error level. d = 0.06.

Substituting into the above formula, we caculated sample size with n = 109. In fact, 119 infertile women infected with CT have been described clinical and paraclinical features. To obtain 119 infertyle women infected with CT, A total of 761 female patients who came for examination and treatment of infertility at the NHOG from January 2020 to December 2021 were selected for the study.

- Sample selection: we used convenience sampling method.Particularly, during the research period from January 2020 to December 2021, infertile women who came for examination and treatment at the NHOG were examined and screened. Those who had enough the inclusion criteria were examined and took endocervical samples to identify CT, and were interviewed to collect information.

2.1.5. Research content

- To determine the rate of CT infection in infertile women.
- To describe the clinical characteristics of CT infection in infertile women.
- To describe the paraclinical characteristics of genital tract CT infection.
- To analyze some factors related to CT infection of genital tract on women.

2.1.6. The research criteria

Research criteria include: age, age group, occupation, ethnicity, educational level, marital status, mariageable age, age at the first pregnancy, the number of pregnancy, the number of birth, the number of living children, sexual intercourse before marriage, the number of partner who have ever had sexual intercourse, history of abortion, history of miscarriage, history of vaginitis, genital infection, type of ìnertility, duration of infertility, increased vaginal discharge, lower abdominal pain, vaginitis, cervicitis, genital itching, abnormal bleeding outside of menstruation, vaginal burning, dysuria, painful urination, decreased sexual pleasure, abdominal pain outside of menstruation, fever, etc.

2.1.7. Techniques used in research

2.1.7.1. Techniques for investigating information of participant

Participants were explained the purpose, benefits of participating in the study and how to answer the questions. After confirming consent to participate in the study and answering questions in the form, patients are guided the next steps of treatment for infertility.

2.1.7.2. Techniques for gynecological examination and endocervical sample collection

Infertile women whohad enough the inclusion criteria would explain and exam clinical. Then, they would take endocervical sample by an obstetrician or midwife/technician according to standard procedures established by NHOG.

2.1.7.3. Determination of C. trachomatis infection in endocervical sample

endocervical samples were determined to be infected with CT by using the Cobas 4800® CT/NG test kit on the Cobas® 4800 system based on the realtime PCR principle according to the manufacturer's instructions.

2.1.7.4. Microbiological techniques

All techniques were done according to the instructions of the Ministry of Health and standard procedures developed by NHOG.

2.1.7.4. Imaging diagnostic techniques

Including abdominal ultrasound techniques, obstetric and gynecological ultrasound techniques and hysterosalpingography techniques. These techniques are carried out according to the instructions of the Ministry of Health and standard procedures developed byNHOG.

2.1.7.5. Storage and preservation of samples

Endocervical samples in Cobas® PCR Media sample preservation solution (Roch) are labeled with codes and necessary information including: date and time of sample collection, name, age and stored at -20 ^{0}C.

2.2. Participant, period, location and methods for the second objective: to determine the genotype of C. trachomatis isolated from participant

2.2.1. The participant

- The participants: infertile women infected with CT who were selected for the first objective and CT bacteria.

- Sample selection criteria for genotyping determination: The CT-infected patient sample had a PCR product with only one clear band, size of 1100bp and the good sequence.

***2.2.2. Research location*:** National Hospital Of Obstetric And Gynectoogy and Vietnam Military Medical University.

***2.2.3.The Period*:** 1/2020 to 12/2022.

2.2.4. Study design and sample size

2.2.4.1. Research design: Cross-sectional descriptive research with analysis.

2.2.4.2. Sample size and sampling method

- Including 81 samples with good sequences collected from 119 analyzed samples.
- Sample selection method: Among 119 CT-positive endocervical samples, only 81 samples had good sequences for genotyping analysis.

2.2.5. Research content

- To determine the frequency of CT genotypes isolated from participants.
- To determine the nucleotide similarity ratio of CT genotypes isolated from participant with data on the gene bank.
- To build the pedigree and analyze the ompA gene polymorphism characteristics of CT.
- To analyze the relationship between CT genotype and some characteristics of the participant.

***2.2.6. The reseach criteria*:** CT genotype, the number of nucleotide differences, nucleotide change at a position on the nucleotide chain, type of amino acid change.

2.2.7. Techniques used in research

2.2.7.1. Techniques for DNA extraction and test

AND of endocervical sample was extracted y using the QiAamp® DNA mini kit (QIAGEN, Code 51304, Germany). The obtained DNA was stored at -20 ^{0}C until further analysis.

2.2.7.2. Amplification of CT ompA gene by semi-nested PCR reaction

In this study, to increase the sensitivity of the ompA gene amplification reaction, the semi-nested PCR technique was used as described in a previous study conducted by Beni and colleagues (2010). .

2.2.7.3. Gene sequencing technology

The second round PCR product with good quality was sent to First BASE Laboratories Sdn Bhdservice (Kembangan 43300, Selangor, Malaysia) for purification and sequencing with 5 primers: PCTM3 (5'-TCC TTG CAA GCT CTG CCT GTG GGG AAT CCT-3'), CT5 (5'-ATT TAC GTG AGC AGC TCT CTC AT-3'), CT3 (5'-ACT TTG TTT TCG ACC GTG TTT TG-3'), CT4 (5'-GAT TGA GCG TAT TGG AAA GAA GC-3') and self-designed primer CT789 (5'-TGC CTC TAT TGA TTA CCA TG-3'). The ompA gene sequence was concatenated, joining the obtained sequences of 5 primers using spermatological tools. Then, the obtained ompA gene sequence was compared with the reference sequences on the gene bank using the BLAST tool to determine the genotypes and gene polymorphisms of CT.

2.3. Inputing data, analyze and process data.

- The results were coded, analyzed, and processed by using IBM SPSS software version 20.0. Criteria were calculated as percentages and/or averages. Related factors were identified through p-value analysis, OR odds ratio, and 95%CI. A p value < 0.05 was determined to be statistically significance.

- The ompA gene sequence of CT was analyzed and edited with bioinformatic software Mega 7.0.9, Bioedit 7.2.5 and compared with data on the gene bank using the BLAST tool (http://blast .ncbi.nlm.nih.gov/Blast.cgi) for genotyping. Reference ompA gene sequences include: DQ064280 (genotype B); X62919 (genotype D); X52557 (genotype E); X52080 (genotype F); CP001888 (genotype G); X16007 (genotype H); DQ116397 (genotype Ia); JN795432 (genotype J); JN795430 (genotype K). The family tree was built using Mega 7.0.9 software, with a boostrap factor of 1,000 repetitions. The ompA sequence of Chlamydia caviae (KY777669.1) was used as an extraspecific reference.

2.4. Error and error elimination

The data was recorded carefully, meticulously, accurately and cleaned before inputting. Practioners were trained and understood the technical sampling process and sample requirements. After being collected, the sample is immediately transferred to the microbiology department for testing on the Cobas® 4800 automatic system. Positive samples were stored and preserved carefully. PCR reactions were controlled with negative and positive controls.

2.5. Ethnicity of research

The study was approved by the Ethic Council in biomedical research at the NHOG according to Decision No. 221/QD-PTSW dated March 5, 2020 and the National Institute of Malaria - Parasitology – Entomology according to Decision No. 182/QD-VSR dated February 24, 2020. The clinical examination process for patients is carried out in a closed room with a minimum of 2 medical staffs. All participants were informed about the benefits and purposes of the study. Information about the research subject's medical condition and personal information are kept confidential and are only used for research purposes.

Chapter 3. THE OUTCOMES

3.1. The common features of 761 infertile women with *Chlamydia trachomatsis* infection

The majority (70,96%) of woman going to the NHOG to treat for infertility were over 25 years old, the mean age was 29.29 ± 5.95 (19 - 52). 93.96% was Kinh. There were 46 infertile woman (6.04%) was ethnic minority. The proportion of infertile woman who was official and bussiness was highest (28.12% và 28.65%). The majority of them had high or higher education level (89.62%).

Table 3.1. The characteristics of obstetric history of patients (n = 761)

History		The number	The rate %
Vaginitis, cervicitis	Yes	283	37.19
	No	478	62.81
Caesarean	Yes	62	8.15
	No	699	91.85
Vaginal operation, abdominal operation and pelvic operation	Yes	127	16.69
	No	634	83.31
Abortion	Yes	181	23.78
	No	580	76.22
Miscarriage	Yes	208	27.33
	No	553	72.67
Ectopic pregnancy	Yes	27	3.55
	No	734	96.45
The number of partner	One	562	73.85
	More than one	199	26.15
Partner with STDs	Yes	12	1.58
	No/Unidentify	749	98.42

The percentage of infertile woman got vaginitis or cervicitis was 37.19%; The figures for caesarean and miscariage were 8.15% and 27.33%, respectively. The proportion of lower abdominal and pelvic opertation was 16.69%; the figure for abortion and ectopic pregnacy were 23.78% and 3.55%, respctively. The rate of woman having sex with more than one partner was 26.15%; The figure for infertile woman had partner with STDs was 1,58%.

The mean age for menstruatation was 14.31 ± 1.54, the youngest was 10 and the oldest was 21. The mean age for the first sexual intercourse was 21.74 ± 3.64, the youngest was 15 and the oldest was 37. The mean age for marriage was 23.73 ± 4.06, the youngest was 16 and the oldest was 44.

Table 3.2. The characteristics of sexual intercourse and marriageable age (n = 761)

Features		The number	The rate %
The age for the first sexual intercourse	Under 18	154	20.24
	Over 18	607	79.76
The time for the first sexual intercourse	Before marriage	443	58.21
	After marriage	318	41.79
The number of partner	More than one	199	26.15
	Only one	562	73.85
The age of marriage	Under 22	233	30.62
	Over 22	528	69.38

The percentage of the the first sexual intercourse of the under18 infertile woman was 20.24%.There was 58.21% had sexual intercourse before getting married and 26.15%

having sex with more than one partner and 30.62% got married when they were under 22 years old.

Table 3.3. The types and the period of infertility (n = 761)

Features		The number	The rate %
The type	Primary infertile	382	50.20
	Secondary infertile	379	49.80
The period	Under 2 years	478	62.81
	2 – 5 years	237	31.14
	Over 5 years	46	6,.4

A total of 761 infertile woman, the percentage of primary infertile was 50.2% and the figure for secondary was 49.8%. The majority of patients (62.81%) went to exam and treat in the first two years.

3.2. The of clinical anf paraclinical characteristics and the related factors to CT infection of genital tract comming to exam and treat ar NHOG

3.2.1. The proportion of infertile woman infected with CT

Of the total 761 patients, there were 119 positive patients. The percentage of patients infected with CT was 15.6%.

3.2.2. The clinical characteristics of infertile woman infected with CT

In infertile woman infected with CT, the percentage of increased vaginal discharge was 67.22%. The percentage of patients with vaginal itching, vaginal irritation, dysuria and decreased feeling of sexual intercourse flutuated from 15.13% đến 21.01%. There were 5.88% getting vaginal bleeding.

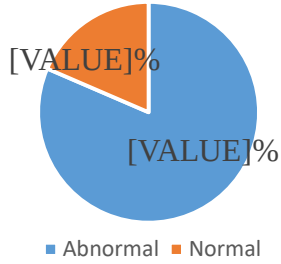


Figure 3.1. The change of vaginal odor of infertile woman infected with CT (n = 81)

Of the total 81 infertile woman shared about vaginal odor, 33 patients (49.74%) had unusual odor, the remain (59.26%) was normal. Vaginal discharge of infertile woman with CT was usually milky white (76.54%). The percentage of yellow – green was 18.52% and the figure for no-color was 4.94%. There was no infertile woman with CT had brown vaginal discharge or other colors.

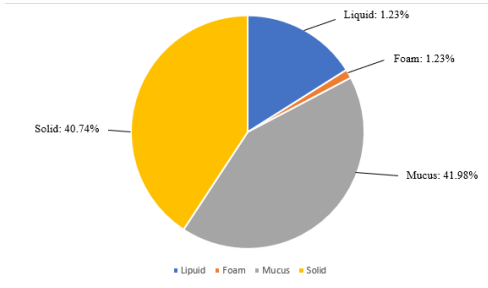


Figure 3.2. The feature of vaginal discharge of infertile woman with CT (n = 81)

Infertile woman infected with CT mostly had mucous and thick vaginal discharge (41.9% and 40.74%). The percentage of infertile woman infected with CT had vaginitis and cervicitis were 75.63% and 80.67%.

Lower abdominal pain outside of menstruation (40.34%) and sore throat (40.34%) were two the most common symptoms. Fever, arthititis and sore eyes ranged from 5.88% to 7.56%.

Table 3.4. The diference of frequency of some symptoms between infertile woman with and without CT (n = 761)

Symptoms	CT (n = 119)		Non CT (n = 642)		*p*
	n	%	n	%	
Increased vaginal discharge	80	67.22	338	52.65	0.003
Vaginal itching	25	21.01	66	10.28	0.001
Abnormal vaginal bleeding	7	5.88	2	0.31	< 0.001
Vaginal irritation	18	15.13	13	2.02	< 0.001
Cricket, dysuria and painful urination	18	15.13	13	2.02	< 0.001
Decreased feeling of sexual intercourse	20	16.81	25	3.89	< 0.001
Vaginitis	90	75.63	202	31.46	< 0.001
Cervicitis	96	80.67	104	16.20	< 0.001
Lower abdominal pain outside of menstruation	48	40.34	64	9.97	< 0.001
Fever	9	7.56	1	0.16	< 0.001
Arthititis	7	5.88	0	0	-
Sore eyes	9	7.56	0	0	-
Sore throat	38	31.93	4	0.62	< 0.001

The proportion of clinical symptoms of infertile woman with CT was higher than the infertile woman without CT. The difference was significant with $p < 0.05$.

3.2.3. The paraclinical characteristics of infertile woman infected with CT

In infertile woman infected with CT, there was 5 patients got hepatitis B. There was no patient with gonorrhea, syphilis and whipworm.

Table 3.5. The proportion of types of bacteria in infertile woman with CT (n = 105)

Causes	The positive	The rate %
Gram – positive bacilus	79	75.24
Gram – positive cocci	24	22.86
Gram – negative bacilus	69	65.71
Candida alblicans	9	8.57
Gram positive bacilus and gram positive cocci	13	12.38
Gram positive bacilus and gram negative bacilus	46	43.81
Gram positive bacilus and Candida alblicans	7	6.67
Gram positive cocci and gram negative bacilus	17	16.19
Gram positive cocci and candida alblican	2	1.90
Candida alblican and gram negative bacilus	3	2.86
One or/and more than one microorganism	105	100

All of infertile women infected with CT were co-infected with at least 1 of the agents including gram positive bacilli, gram positive cocci, gram negativebacilli or candida alblican. In which, the percentage of gram positive bacilli was highest (75.24%), followed by gram negative bacilli (65.71%), gram positive cocci (22.86%) and candida alblican (8.57%).

The test also showed that 2/31 (6.45%) patients had abnormalities on cervical cytology smear; 4/5 patients were positive with HPV.

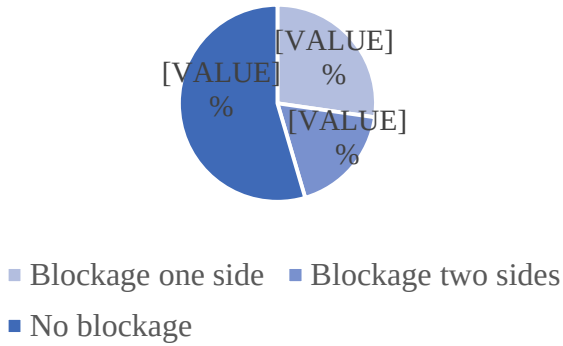


Figure 3.3. The rate of fallopian tube blockage in infertile women with CT (n = 33)

Among 33 infertile women infected with CT who had a hysterosalpingogram, there were 9 patients with unilateral blockage (27.27%), 6 patienst with bilateral blockage (18.18%), and 18 patients (54.55%) had no blockage.

Ultrasound results of 119 infertile women infected with CT showed that 45 people had abnormalities in the uterus and adnexa, accounting for 37.82%. Common abnormalities on ultrasound were polycystic ovaries, abnormal masses in the uterus and fluid retention of fallopian tube.

3.2.4. Some factors related to infectionof genital tract in infertile women

*** Relation between some demographic characteristics and status of CT infection**

Analysis results showed that there was no relation between age, occupation and education level with the rate of CT infection in infertile women.

*** Relation between some historical factors and CT infection status**

Table 3.6. Relationship between some obstetric and gynecological history factors with CT infection in infertile women (n = 761)

History factors		Status of CT infection		*p*	OR (95% CI)
		Positive	Negative		
Vaginitis and/or cervicitis	Yes	81	202	< 0.0001	4.64 (3.05 – 7.07)
	No	38	440		
Caesarean	Yes	36	145	0.0725	1.49 (0.96-2.29)
	No	83	497		
Abortion	Yes	33	175	0.9154	1.02 (0.66 – 1.59)
	No	86	467		
Ectopic pregnancy	Yes	8	19	0.042	2.36 (1.01-5.53)
	No	111	623		
Lower abdominal operation and pelvic operation	Yes	28	99	0.0307	1.69 (1.05 – 2.71)
	No	91	543		

Univariate analysis results showed that there was an relation between history of vaginitis, ectopic pregnancy, lower abdominal operation and pelvic operation with CT infection status. There was no relation between history of abortion and miscarriage and CT infection.

The rate of CT infection in infertile women whose partner has ever had an STD was 5.63 times which was higher (95%CI: 1.78 - 17.76) than in infertile women whose partner has never had the disease. This difference is statistically significant (p = 0.0032).

Table 3.7. Relation between some characteristics of sexual intercourse and CT infection in infertile women

Features	Status of CT infection		*p*	OR (95% CI)
	Positive	Negative		
Age of the first sexual intercourse				
Under 18	45	109	< 0.0001	2.97 (1.95 – 4.54)
Over 18	74	533		
Time of the first sexual intercourse				
Before marriage	83	360	0.0059	1.81 (1.19 – 2.75)
After marriage	36	282		
The number of partner				
More than one partner	45	154	0.0018	1.93 (1.28 – 2.91)
Only one partner	74	488		
Total No	**119**	**642**		

The rate of CT infection in women who had sex for the first time before the age of 18 was 2.97 times higher (95%CI: 1.95 - 4.54) than in women who had sex after the age of 18 (p < 0.0001). The rate of CT infection in sexually active women who had sex before marriage was1.81 times higher (95%CI: 1.19 - 2.75) than in sexually active women who started sexual intercourse after marriage (p < 0.05). The rate of CT infection in infertile women who had sex with more than one person was 1.93 times higher (95%CI: 1.28 - 2.91) than in VS women who have sex with only one person (p < 0.05).

Table 3.8. Relation between marriageable age and CT infection in infertile women

The marriageable age	Status of CT infection		*p*	OR (95% CI)
	Positive	Negative		
Under 22 (< 22 years old)	51	182	0.0018	1.90 (1.27 – 2.83)
Over 22 (≥ 22 years old)	68	460		
Total	**119**	**642**		

The percentage of CT infection in infertile women got married before the age of 22 was 1.90 times higher (95%CI: 1.27 - 2.83) than infertile women got married after the age of 22, the difference was statistically significance (p < 0.05).

*** Relationship between type of infertility, duration of infertile and status of CT infection**

Table 3.9. Relation between type of infertility and CT infection in infertile women

Types of infertility	Status of CT infection		*p*	OR (95% CI)
	Positive	Negative		
The primary	57	325	0.58	0.9 (0.61-1.33)
The secondary	62	317		
Total	**119**	**642**		

Univariate analysis results showed that there was no relation between type of infertility and CT infection (p> 0.05).

CT infection in infertile women dit not relate to infertile detection time.

*** Relation between some clinical characteristics and C. trachomatis infection status in infertile women**

Table 3.10. Relation between vaginitis, cervicitis, lower abdominal pain and CT infection in infertile women (n = 761)

Vaganitis, cervicitis	Status of CT infection		*p*	OR (95% CI)
	Positive	Negative		
Increased viganial discharge				
Yes	80	338	0.003	1.84 (1.22 – 2.79)
No	39	304		
Vaginitis				
Yes	90	202	< 0.0001	6.76 (4.31 – 10.61)
No	29	440		

Cervicitis				
Yes	96	104	< 0.0001	21.59 (13.08 – 35.64)
No	23	538		
Lower abdonimal pain				
Yes	48	64	< 0.001	6.11 (3.90-9.56)
No	71	578		
Total No	**119**	**642**		

The proportion of CT infection in infertile women with signs of increased vaginal discharge, vaginitis and cervicitis was 1.84 times higher (95%CI: 1.22-2.79), 6.76 times higher (95%CI: 4.31 - 10.61) and 21.59 times (95%CI: 13.08 - 35.64) compared to infertile women without these symptoms, the difference wwas statistically significance ($p < 0.05$). The rate of CT infection in infertile women with lower abdominal pain was 6.11 times higher (95%CI: 0.61-1.33) than that of infertile women without this symptom ($p < 0.05$).

Univariate analysis results also showed that, the figure for infertile women with fallopain tube was 1.91 times higher (95%CI: 0.93 - 3.94) than that of infertile women without fallopain tube obstruction. However, the difference is not statistically significance ($p > 0.05$).

Table 3.11. The result of multivariate analysis of the relation between some factors and CT infection in infertile women

Analysis factors	OR (95%CI)	p
History of vaginitis and cervinitis	1.59 (0.91-2.78)	0.106
History of ectopic pregnacy	**3.03 (1.03-8.91)**	**0.043**
History of lower abdominal and pelvic operation	1.16 (0.61-2.22)	0.646
Partner with STDs	1.61 (0.35-7.49)	0.546
The age of the first sexual intercourse (Under 18 years old)	**2.47 (1.29-4.73)**	**0.007**
The time of sexual intercourse (before marriage)	1.22 (0.66-2.23)	0.527
Sexual intercourse with more than one partner	**1,93 (1.05-3.57)**	**0.035**
Under 22 year – old marriage	0.98 (0.54-1.77)	0.979
Increased vaginal discharge	**2.58 (1.38-4.81)**	**0.003**
Vaganitis	1.81 (0.91-3.60)	0.092
Cervicitis	**20.09 (10.08-40.03)**	**< 0.001**
Lower abdonimal pain	**5.61 (3.06-10.31)**	**< 0.001**

Multivariate analysis results showed that factors such as history of ectopic pregnancy, under – 18 sexual intercourse, sexual intercourse with multiple partners, increased vaginal discharge, cervicitis and lower abdominal pain were related to CT infection in infertile women.

3.2. Genotype of C. trachomatis isolated from participants

3.2.1. The result of determining genotypes and analyzing ompA gene polymorphisms

Ninety out of 119 samples which had good quality of PCR2 were sent for

sequencing (Figure 3.4). As a result, 81 samples obtained good and clear sequences which qualified for genotype analysis. There were no samples infected with a combination of two genotypes.

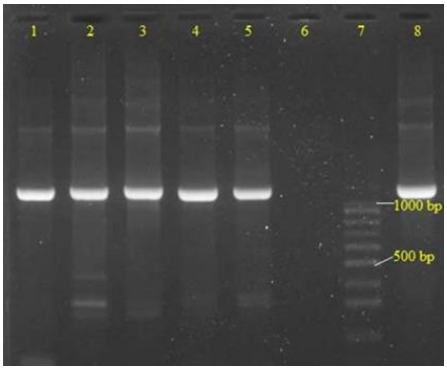
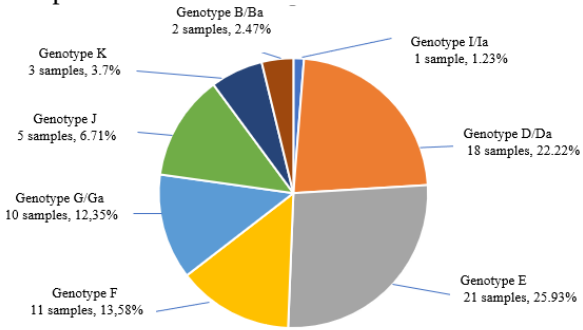


Figure 3.4. The product of the second round of PCR reaction amplified the ompA gene of CT from the endocervical sample

In Figure 3.4, the wells 1-5: ompA gene amplification products of CT-infected endocervical samples with corresponding genbank codes MZ407931, MZ407932, MZ407933, MZ407934 and MZ407935; well 6: negative control; 7-month well of standard DNA 100 - 1000 bp; well 8: positive control.



Hình 3.5. The percentage of C. trachomatis genotypes (n = 81)

The results showed that 9 different genotypes were identified, including B/Ba, D/Da, E, F, G/Ga, H, I/Ia, J and K. Among them, Genotype E accounted for the highest proportion (n = 21; 25.93%), followed by genotypes D/Da (n = 18; 22.23%), F (n = 11; 13.58%).), G/Ga (n = 10; 12.35%), J (n = 10; 12.35%), H (n = 5; 6.17%), K (n = 3; 3.70%), B/Ba (n = 2; 2.47%) and the lowest was I/Ia (n = 1; 1.23%). Details in Figure 3.5.

The 81 analyzed CT ompA gene sequences had a high nucleotide similarity rate compared to reference sequences on the gene bank, ranging from 99.10% to 100%. The resulting genotypes B/Ba, D/Da, H, I/Ia, J and K had 1 to 10 nucleotide differences compared to the reference sequences, with 19 change positions detected, of which 7 nucleotide changed lead to amino acid substitutions. Among these 81 sequences, 22

sequences of 9 genotypes of the ompA gene were registered and assigned codes on GenBank with codes from MZ407931 to MZ407947 and from OP899639 to OP899643.

3.3.2. Relation between genotypes and some characteristics of patients

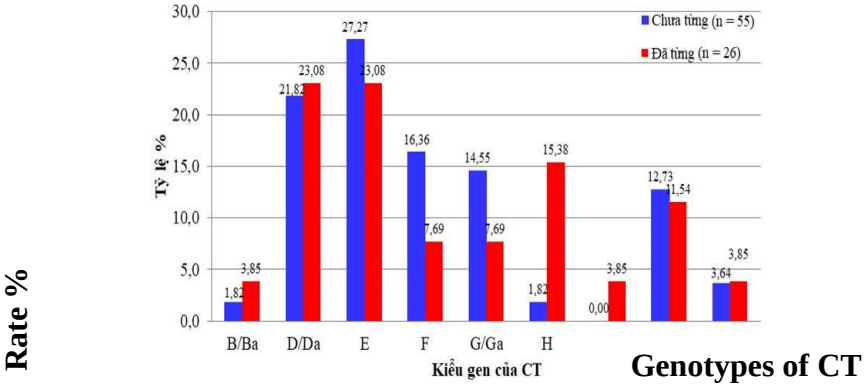


Figure 3.6. Distribution of CT genotypes according to miscarriage history

Analysis results showed that the rate of genotype H in women who had miscarriage was significantly higher than that of women without miscarriage (15.38% compared to1.82%, p = 0 ,0186). Other genotypes did not different between the two groups.

Table 3.12. Distribution of CT genotypes according to infertile type (n = 81)

Genotype	Types of infertile [n (%)]		*p*
	Primary	Secondary	
B/Ba	1 (2.70)	1 (2.27)	0.9017
D/Da	7 (18.92)	11 (25.00)	0.5147
E	10 (27.03)	11 (25.00)	0.8365
F	9 (24.32)	2 (4.55)	**0.0101**
G/Ga	4 (10.81)	6 (13.64)	0.7015
H	1 (2.70)	4 (9.09)	0.2368
I/Ia	0 (0)	1 (2.27)	-
J	4 (10.81)	6 (13.64)	0.7015
K	1 (2.70)	2 (4.55)	0.6626
Total No	**37 (100)**	**44 (100)**	

Genotype F which was found in the primary infertility group was significantly higher than that of the secondary infertility group (24.32% compared to 4.55%, p = 0.0101). Other genotypes did not different between these two groups.

Analysis results also showed that in infertile women, the rate of genotypes was not different between two age groups, with and without a history of vaginitis, sexual intercourse before and after the age of 18, sexual intercourse before marriage and after marriage, with and without vaginitis/cervix, with and without lower abdominal pain, with and without fallopian blockage,etc.

Chapter 4. DISCUSSION

4.1. Some common characteristics of 761 infertile women infected with CT

In this study, we collected information and tested endocervical sample of 761 infertile women coming for examination and treatmnet at NHOG to describe the clinical and paraclinical characteristics as well as identify related factors of infertile women infected with CT. As a result, there were 119 infertile women infected with CT, accounting for 15.6%. The mean age was 29.29 (19-52), the majority were over 26 years old (70.96%) and the majority were Kinh people (93.96%). The percentage of patients working in business, trading and official were highest (28.65% and 28.12%). Most of patients had high or higher education level (89.62%).

4.2. Clinical and paraclinical characteristics and some factors related to CT infection in infertile women

4.2.1. The rate of CT infection in infertile women

In this study, Of the total 761 infertile women tested by real-time PCR using the Cobas® CT/NG biological product (Roch), there were 119 infertile women infected with CT, making up 15.6%. This rate is quite high compared to previously published studies. It was also much higher than women who were asymptomatic, pregnant or uninfertile. Particularly, CT infection rates typically ranged from 2% to 17% in women who were asymptomatic. The figure for pregnant women ranged from 4.6-18%, usually less than 10%.

In Vietnam, there are a few studies on CT infection in infertile women. Nguyen Hai Dang and et al (2020) conducted research on 541 cases of infertile female who came for examination and treatment at the Reproductive Endocrinology And Hygiene Center of Hue University of Medicine and Pharmacy Hospital, from June of 2017 to June of showed that the rate of CT infection were 5.7%. These figures were much lower than those of our study (5.7% compared to 15.6%).

Compared with some studies in the world, the rate of women with genital tract CT infection in this study was similar to the infection rate in India (15.7%) and Saudi Arabia (15.0%); higher than in China (5.9%), Turkey (2.15%), Rwanda (3.3%), Jordan (3.9%), Argentina (5.3%), and Malaysia (7.3%), but lower in Palestine (20.2%), Nigeria (28.0%), Netherlands (29.5%), Iran (32.0%), and Tanzania (36.21 %). Variation in infection rates between domestic studies may be due to the detection method used. This has been shown by previous studies around the world.

The results also showed that the rate of CT infection was not different between age groups, occupation, education level, history of abortion, miscarriage, ectopic pregnancy, type of infertility and duration of infertility. But there was a difference between a history of vaginitis, a history of lower abdominal operation, and partner who had a sexually transmitted disease.

4.2.2. Clinical characteristics of CT infection

According to the CDC of United State, Although increased vaginal discharge is an important symptom for diagnosing genital inflammation in women, it is no specific clinical

symptoms. Analysis results on 119 infertile women infected with CT showed that 67.22% had increased vaginal discharge, which was higher than that of infertile women without CT (67.22% compared to 52.65%), the difference was statistically significant ($p < 0.05$). The results in this study were similar to the results of Rawre and et al (2016, but lower than the results of Tran Dinh Vinh and et al (2020) (67.22% compared to 88.5%). This difference may be due to CT co-infection with other microbial cause which were not investigated because of lacking of funding and implementation duration.

In this study, The percentage of abnormal vaginal odor in infertile women infected with CT was 40.74% . According to references, abnormal vaginal odor is a sign of uterine vaginitis, but it is not a specific symptom of any microbial causes. In this study, all of infertile women infected with CT were co-infected with at least 1 other microbial cause leading to vaginitis. Therefore, abnormal vaginal odor only had value to suggest that women had vaginitis, and it was not a specific symptom of CT infection.

The proportion of abnormal vaginal color infertile women infected with CT was 95.06%. The majority was milky white (76.54%). Changes of vaginal discharge features (thick, mucous and foamy) occured in more than 80%. Changes in color and characteristics of vaginal discharge were consistent with the frequency of vaginitis and cervicitis in women infected with CT.

Among 119 infertile female patients infected with CT, genital itching occurred in 21.01% of patients. In this study, The rate of genital itching in infertile women infected with CT was much lower than that of Tran Dinh Vinh and et al (2020) conducted on women examined at the Da Nang Obstetriet and Pediatriet Hospital (21.01% compared to 66.1%). This difference may be the participants in two studies were different. Particularly, this study was conducted on infertile women, including patients with and without clinical symptoms. While the study of Tran Dinh Vinh and et al (2020) was conducted on patients who came for gynecological examination, most of them were patients with clinical symptoms.

According to analysis results, Therer were 9 patients who had abnormal vaginal bleeding, most of them were CT infection (7 patients). Bleeding often originates from the cervix caused by endocervical inflammation. Abnormal bleeding in the genital tract can occur in 30% of women infected with CT. On physical examination, women infected with CT have an enlarged, exposed cervix that easily bleeds when touched. On women who came for gynecological examination at Da Nang Obstetriet and Pediatriet Hospital in the study of Tran Dinh Vinh and colleagues (2020), the rate of symptoms of abnormal bleeding in the genital tract (mid-cycle bleeding) menstruation, bleeding after sexual intercourse) was 41.7%.

Other symptoms such as vaginal burning, dusuria, painful urination and decreased pleasure during sexual intercourse in infertile women infected with CT were also much higher than those of infertile women without CT infection, the difference was statistically significant. statistiet al ($p < 0.05$).

Lower abdominal pain is a fairly common clinical symptom in women infected with CT, especially patients with pelvic inflammatory caused by CT. In this study, the

proportion of infertile women with lower abdominal pain was only 14.72%. The result was quite similar to the outcome of Kamel (2013) which also conducted on infertile women in Saudi Arabia (14.72% compared to 15%). In addition, in this study we also received some systemic symptoms such as fever, arthritis, sore eyes and sore throat. The rate of these symptoms in infertile women infected with CT was higher than those of infertile women without CT infection, the difference was statistically significant.

Clinical manifestations of infection with sexually transmitted microorganisms in general and CT in particular are often insignificant and less specific, especially in women. Because there are no specific symptoms, screening in high-risk subjects is very important. According to recommendations from the CDC of the United State, all sexually active women who are under 25 years of age should be screened for CT annually. For older women with risk factors, annual screening is also recommended.

4.2.3. Paraclinical characteristics of CT infection

Test results of some microorganisms in the vagina showed that the proportion of gram positive bacilli was 75.24%, the figures for gram negative bacillus, gram positive coccus and Candida alblican were 65.71%, 22.86% and 8.57%, respectively. The rate of co-infection with at least one of the above agents was 100%. Analysis results showed that although the rate of infection with gram positive bacilli, gram negative bacilli, gram positve cocci and Candida alblican in infertile women infected with CT were quite high, it was not related to increased vaginal discharge, genital itching or abnormal vaginal odor. This showed that these symptoms were not specific to any genital infectious agent. Many studies also showed the similar result.

In this study, all of 119 infertile women infected with CT were given ultrasound to investigate abnormalities in the uterine adnexa. The results showed that 37.82% had abnormal images. Most abnormal images appeared in the uterus, fallopian tube and ovaries. Ultrasound gave indirect results about abnormalities in the uterine adnexa and was an important guide to find the cause of infertile. Ultrasound is also a supporting tool in performing treatment interventions for infertile such as egg monitoring, egg collection and embryo transfer. Along with ultrasound of the uterine adnexa, 31 patients infected with CT had an scan of fallopian tube, resulting in 15 patients with obstruction (45.45%), of which 9 had single obstruction (27.27%) and 6 had the couple (18.18%). Although the number of patients receiving scans of fallopian tube was not large, the number of patients with obstruction was quite high. This result was consistent with the findings on ultrasound. Research by Ho Van Phuc and et al. (2021) on 122 cases of infertile women infected with CT found that the rate of fallopian tube occlusion was 77.9%, higher than our study (77.9% compared to 45%).

4.2.4. Some factors related to CT infection in infertile women

In this study, the rate of CT infection of under 25 - year old infertile women was higher than over 25 - years old group, but the difference was not statistically significant ($p = 0.9227$). Previously published studies agreed that the rate of CT infection is often higher in younger women. Research by Rawre and colleagues (2016) on infertile women in India showed that the rate of CT infection in under 30 – year old infertile women was higher than

over 30 - year old group, but the difference was not statistically significant (OR = 1.25; 95%CI: 0.73–2.15). Another study by Li and et al (2021) conducted in Guangdong, China also showed the similar results. Previous studies agreed that the young is a high risk factor for CT infection and this group should be prioritized in health education, behavior change and screening programs for CT infection, especially in the community. The Preventive Services Task Force and the CDC of United State recommend that all women who were under 25 years older had sex and older women with risk factors should be screened for CT infection annual. However, the issue of routine screening for CT infection has not really received attention in Vietnam. Recently, the Ministry of Health issued "Guidelines for screening and treatment of Chlamydia trachomatis and gonococcal infections in pregnant women" (2019) and "Guidelines for diagnosis and treatment of Chlamydia trachomatis" (2021). However, only women who are in the first 3 months of pregnancy age under 25 or over 25 years old with high risk are recommended for routine screening, the remain is not recommended for routine screening.

Multivariate analysis results showed that there was no relation between history of abortion, miscarriage, type of infertility, duration of infertility, partner with a history of STDs, history of vaginitis, cervicitis, lower abdominal surgery, sexual intercourse before marriage, marriageable age are under 22 years old, symptoms of vaginitis with CT infection, etc.

Having sex with multiple partners is believed to increase the risk of CT infection. In this study, CT infection rate was related to age at first sexual intercourse and the number of partner. Specifically, the proportion of infertile women who started having sex before the age of 18 infected with CT was 2.47 times higher (95%CI: 1.29-4.73) than the group who started having sex after the age of 18, the difference was statistical significance ($p = 0.007$). The group of infertile women who had sex with more than 1 partner had a CT infection rate 1.93 times higher (95%CI: 1.05-3.57) than those who had sex with only 1 partner ($p = 0.035$). This result was consistent with published medical literature.

This study also showed that increased vaginal discharge was associated with CT infection in infertile women. Specifically, the rate of CT infection in women with symptoms of increased vaginal discharge was 2.58 times higher (95%CI: 1.38-4.81) than that of women without this symptom. The difference was statistically significant ($p = 0.003$). Research by Rawre and et al (2016) on infertile women in India and by El Qouqa and et al (2009) in Palestine also showed the similar results. According to the study of Rawre et al. (2016), the rate of CT infection in women with symptoms of increased vaginal discharge was 14.58 times higher (95%CI 8.28–25.68) than that of women without this symptoms ($p < 0.001$). Cervicitis and lower abdominal pain were also determined to be associated with CT infection in infertile women. Specifically, the rate of CT infection in infertile women with cervicitis and lower abdominal pain were 20.09 times higher (95%CI: 10.08 - 40.03) and 5.61 times higher (95%CI: 3). .06-10.31) compared to infertile women with these symptoms, the difference was statistically significant ($p < 0.001$).

4.3. Genotypes of CT in infertile women

4.3.1. Genotypes and polymorphisms of the ompA gene of CT

In this study, 9 different genotypes of CT were identified, including E, D/Da, F, G/Ga, J, H, K, B/Ba and I/Ia. Among them, the rate of genotype E was highest proportion (25.93%), followed by D/Da (22.22%) and F (13.58%), while genotypes G/Ga, J, H, K, B/Ba and I/Ia had lower rates, ranging from 1.23% to 12.35%.

Three genotypes E, D and F were found to predominate in infertile women in India (47.8% E, 32.2% D and 20% F), and symptomatic women in Argentina (46.9% E, 32.2% D and 20% F). %E, 21.0% D, and 16.1% F). In some other countries, these three genotypes of CT were also the most commonly reported in the genitourinary tract. However, in some places the distribution of genotypes had some differences from our study. Specifically, Genotypes D, F and K were the most common in patients with sexually transmitted diseases in Thailand, while genotype F predominates in Mexico and Brazil. Genotypes D and J were most common in Guanxi and Guangzhou, China, with rates of 29.69% and 28.6%, respectively. According to some documents, the reason for this difference was to geographical distribution, characteristics of the population and period. To learn more about this issue, more research is needed on the frequency and distribution of CT genotypes in different subjects and geographical areas.

In Vietnam, Pham Dang Bang and colleagues (2011) used PCR-RFLP technique to determine CT genotypes in 85 patients examined at the National Dermatology Hospital from January 2010 to August 2010 showed that three genotypes D/Da, E, F also predominate with over 80% of the total cases identified. However, it was different from our study; Particularl, in the study of Pham Dang Bang and colleagues (2011), genotype D accounted for the highest proportion with 29.5%, followed by genotype E (27.1%) and ranked third was genotype F (23.5%), while in our study genotype E accounted for the highest proportion (25.93%), followed by D/Da (22.22%) and F (13.58%). Research by Pham Dang Bang and colleagues (2011) also showed that there was a difference in the distribution of genotypes between the under 25 year old age group and the other group. While in our study, There was no difference about the distribution of genotypes between age groups . This may be due to the study of Pham Dang Bang and colleagues (2011) conducted on both men and women infected with CT who came to be examined for sexually transmitted diseases at the National Dermatology Hospital, while we only studied on infertile women.

In this study, 19 nucleotide changes (point mutations) were detected in genotypes B/Ba, D/Da, H, I/Ia, J and K, of which 7 nucleotide changes leading to amino acid substitution (missense mutation). Genotype B had the highest number of point mutations with 10 positions, including 3 missense mutations.

These changes were different from the research of Jurstrand and et al (2001) in Sweden and Yang and et al (2010) in Guangzhou, China on patients with sexually transmitted diseases. A recent study by Tang and et al (2022) also conducted in Guangzhou, China in women of childbearing age attending gynecological examinations showed that all 8 identified genotypes had nucleotide changes on the ompA gene with a total of 25 different variable positions. The number of nucleotide changes for each genotype ranged from 1-11, in which genotype H had the most changed positions (11

positions) and most of them lead to amino acid changes (10/11). In infertile subjects, we found very few studies analyzing the ompA gene polymorphism of CT.

4.3.2. Relation between genotype of CT and some characteristics of participant

The analysis results showed that there were no differences in the distribution of genotypes between age groups, history of vaginitis, age of starting sexual intercourse, the time of having sex before and after marriage, with and without vaginitis/cervix, with and without lower abdominal pain, with and without fallopian tube blockage ($p > 0.05$). However, genotype F tended to be higher in infertile patients aged under 25 years compared to infertile patients aged over 25 years. This result was consistent with the results of Gao and et al (2007) on high-risk women in China and Liu and et al (2022) on women of childbearing age in Shenzhen (China) but they were different from the study by Pham Dang Bang and et al (2011) who studied subjects who came to be examined for venereal diseases at the National Dermatology Hospital in 2010.

Different studies showed that the relation between CT genotypes and clinical features in different subjects was very different. In our study, no association was found between D/Da and E genotypes with age group, history of vaginitis, age and time of sexual intercourse as well as some clinical characteristics. However, some studies found an association with some clinical characteristics. For example, in the study of Casillas-Vega et al (2017), genotype D was more common in women who had a genital infection during a previous pregnancy, and genotype E had a higher prevalence in women who who had an ectopic pregnancy and had green vaginal discharge. According to the study by Tang and colleagues (2022) on women examined at Guangdong Women's and Children's Hospital (Guangzhou, China), D/Da genotype infection increased the number of white blood cells in urine compared to other genotypes.

Regarding to genotype F, the infection rate of this genotype in the primary infertility group was significantly higher than that of the secondary infertility group (24.32% compared to 4.55%, $p = 0.0101$). According to our knowledge, there have been no studies analyzing the relation between F genotype and type of infertility. The result was a new point in this study. Some studies showed that the F genotype was found to be quite high in unmarried patients, pregnant women or patients with increased vaginal discharge or genital itching.

Redarding to G genotype, the research by Gao and et al (2007) in China on women examined for sexually transmitted diseases showed that the G/Ga genotype was related to the abdomimal pain, but its ws not related to increased abnormal vaginal discharge. Specifically, according to this study, 54.5% of women with lower abdominal pain were infected with genotype G, while the figure for patients without lower abdominal pain was only 5.2%, the difference was statistically significant. $p < 0.05$. Duynhoven and et al (1998) also showed that the the rate of G/Ga genotype was up to 32%, significantly higher than other genotypes in patients with symptoms of lower abdominal pain. Research by Tang and colleagues (2022) showed that the identified G genotype was related to increased abnormal vaginal discharge but was not related to lower abdominal pain. Analysis results from our study showed that there was no relation between genotype G and

symptoms of lower abdominal pain as well as increased vaginal discharge in infertile women. The different results between studies suggested that the role of genotype G in clinical symptoms needs to be further elucidated.

Regarding to genotype H, we found that the rate of genotype H in women with a history of miscarriage was significantly higher than in women without this history (15.38% compared to 1.82%, p = 0.0186). According to research by Liu and colleagues (2022) on women of childbearing age in Shenzhen (China), there was no relationship between genotypes and clinical manifestations in the genital tract, but genotype H was related to the appearance of cytological abnormalities in the cervix (p = 0.006; aOR = 8.16, 95%CI: 1.86-36.6). Due to limitations in the scope of the study, we have not surveyed the cervical cytology of infertile women. Therefore, the association between genotypes and cytological abnormalities in the cervix has not been analyzed.

Our study has some limitations: the number of patients undergoing hysterosalpingography was small, so the analysis of the impact of CT on infertility were not clarified. Tests for changes in urine, vaginal and urethral secretions and cervical cytology tests were not fully conducted, so analysis of the relationship between genotype and characteristics of these factors are still open issues. Some clinical characteristics of patients were not been collected and analyzed, so information about the relationship with CT infection was incomplete. The sample size for determining genotypes in general and for each genotype was still small, so the assessment of the relationship between genotypes and the characteristics of the patients was not really accurate. In addition, due to lacking of time and budget, this study only determined genotypes using the ompA gene marker without having the conditions to sequence many different target genes (multi-locus sequencing) to determine identify variations (subtypes) of CT genotypes. However, the research results contributed to supplementing genotypic data and ompA gene polymorphism characteristics of CT isolated in infertile women in Vietnam. This is also valuable reference information for future studies to have more evidence to explain the relationship between CT infection and infertile problems in women.

CONCLUSION

1. Clinical and paraclinical characteristics and some factors related to C. trachomatis infection in infertile women coming for exmination and treatment at NHOG

Through a study of 761 infertile women who came for examination and treatment at NHOG (2020-2022), there were 119 patients (accounting for 15.6%) infected with C. trachomatis. In infertile women infected with C. trachomatis, clinical and paraclinical characteristics and related factors are following:

1.1. Clinical characteristics

Analysis of 119 infertile women infected with C. trachomatis showed:

- Increased vaginal discharge was the most common symptom in infertile women infected with C. trachomatis with 67.22%, followed by abdominal pain outside of menstruation (40.34%), and genital itching (21.01%).), decreased sexual pleasure

(16.81%), vaginal burning (15.13%), painful urination, dysuria (15.13%), and abnormal bleeding outside of menstruation (5.88%) .

- The rates of vaginitis and cervicitis in infertile women infected with C. trachomatis were 75.63% and 80.67%, respectively.

1.2. Paraclinical characteristics

The results of analyzing the paraclinical characteristics of 119 infertile women infected with C. trachomatis showed that:

- 4.20% of infertile women were infected with hepatitis B virus. There were no cases of gonorrhea, syphilis and vaginal flagellum infection.

- 100% of infertile women infected with C. trachomatis were co-infected with at least 1 microorganism belonging to one of the groups of gram positive bacilli, gram negative bacilli, gram positive cocci and candida alblican.

- 45.45% of infertile women infected with C. trachomatis had fallopian tube scans showing fallopian tube blockage.

- 37.82% of infertile women infected with C. trachomatis had abnormal images in the adnexa on ultrasound.

1.3. Some related factors

Some factors related to genital C. trachomatis infection in infertile women including: history of ectopic pregnancy (OR = 4.64; 95%CI: 3.05 - 7.07), sexual intercourse before age 18 (OR = 2.97; 95%CI: 1.95 - 4.54), having sex with many partners (OR = 1.93; 95%CI: 1.28 - 2.91).

2.2. Genotype of Chlamydia trachomatis isolated in infertile women

Genotypic analysis by sequencing of 81 endocervical samples infected with C. trachomatis, the results showed that:

- There were 9 different genotypes identified, including: B/Ba, D/Da, E, F, G/Ga, H, I/Ia, J, and K. Of these, the proprtion of genotype E was the highest, making up 25.93%, followed by D/Da (22.23%), F (13.58%), G/Ga (12.35%), J (12.35%), H (6.17%), K (3.70%), B/Ba (2.47%), and I/Ia (1.23%).

- The rate of genotype H in women with a history of miscarriage was significantly higher than that in women without a history of miscarriage. The rate of F genotype in the primary infertility group was significantly higher than that in the secondary VS group.

RECOMMENDATION

- It is necessary to perform testing to detect C. trachomatis infection in infertile women, especially in infertile women with a history of ectopic pregnancy, sexual intercourse before the age of 18, sexual intercourse with many people, increased vaginal discharge, and cervicitis and lower abdominal pain outside of menstruation.

- It is necessary to test for other genital infectious agents for infertile women infected with C. trachomatis to serve the treatment of genital tract infections and infertility.

- To continue to expand research on determining genotypes and variations of C. trachomatis in infertile women to analyze the role of acquired genotypes in clinical and paraclinical characteristics.

LIST OF PUBLISHED SCIENTIFIC ARTICLES

1. Nguyễn Hòa, Vũ Văn Du, Đỗ Ngọc Ánh, Nguyễn Xuân Kiên (2023). Đặc điểm lâm sàng nhiễm *Chlamydia trachomatis* đường sinh dục ở phụ nữ VS đến khám và điều trị tại Bệnh viện Phụ sản Trung ương (2020-2022). *Tạp chí Y Dược lâm sàng 108*, tập 18 (3/2023), tr: 145-151.

2. Nguyễn Hòa, Vũ Văn Du, Nguyễn Thị Như Quỳnh, Nguyễn Lê Vân, Đỗ Ngọc Ánh (2023). Xác định các kiểu gen và phân tích đa hình gen *omp*A của *Chlamydia trachomatis* phân lập từ đường sinh dục của phụ nữ Việt Nam bị VS. *Tạp chí Khoa học và Công nghệ Việt Nam*, Tập (số), trang: xxxx-xxxx (*Bài báo đã có giấy chấp nhận đăng của Tạp chí*).