

Vaginal Discharge Relationship To Chlamydia Trachomatis (Ct) In Urine Pcr Examination And Hsg Results In Case Of Infertility In The National Hospital Of Timor Leste

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Abstract

Background: The cause of transmission of Chlamydia trachomatis (CT) bacteria tends to occur due to sexual intercourse. Chlamydia trachomatis as one of the causes of vaginal discharge is a common occurrence in sexually active women. Causes of infertility can occur due to women's reproductive health problems, namely problems with ovulation dysfunction, endometriosis, poor nutrition, hormonal disorders, to disruption of sperm transport from the cervix to the fallopian tubes. Examination using PCR has a sensitivity and specificity of the polymerase chain reaction (PCR) method of 91.7% and 99.7%, respectively. Purpose: The purpose of this study was to analyze the relationship between vaginal discharge and Chlamydia trachomatis (CT) in infertility by urine PCR examination at the Timor-Leste National Hospital. **Methods:** This research is an analytical observational study with a cross sectional design. Urine was taken from 30 respondents, tested using PCR. **Results:** Characteristics of respondents showed that the most occupations were in the civil servant group (46.7%) and the highest education was in the SMA and S1 groups (46.7%) and all respondents did not use contraception (100%). Respondents with vaginal discharge 53,3% CT positive and no vaginal discharge 66,7% CT negative. The results of the Fisher's Exact test showed that there was a relationship between HSG and CT . with strong correlation strength ($C = 0.634$; $p > 0.001$). The results of the Fisher's Exact test showed that there was no conclusion relationship with CT $p > 0.05$. **Conclusion:** There is no relationship between vaginal discharge and Chlamydia Trachomatis (CT) in infertility with PCR urine examination at the Timor-Leste National Hospital.

Keywords: Infertility, PCR, Chlamydia trachomatis, Vaginal discharge

1. Introduction

Cases of sexually transmitted infections (STIs) can occur with clinical symptoms or without symptoms (asymptomatic). The cause of transmission of Chlamydia trachomatis (CT) bacteria tends to occur due to sexual intercourse. (Agustini & Arsani, 2015). Based on data obtained from The Centers for Disease Control and Prevention (CDC) in the United States, it is explained that every year this

disease infects about 700,000 people (Arjani, 2015). According to WHO data based on prevalence data in 2016 the estimated global prevalence of CT in women aged 15-49 years is 3.8% and in men 2.7%. In Timor-Leste, the global incidence of Chlamydia trachomatis infection is unknown. Previous research conducted by Integrate Biological and Behavioral Surveillance (IBBS) in 2011 on a population considered at risk, namely the

population of female commercial sex workers in Timor-Leste, the prevalence of Chlamydia trachomatis infection reached 40%. CT bacteria also have the potential to interfere with the female reproductive system, namely infertility and vaginal discharge. WHO states that 75% of women in the world experience vaginal discharge once in their lifetime, while some 45% of women experience vaginal discharge more than twice. Vaginal discharge is a symptom that is often experienced by most women. When vaginal discharge occurs before menstruation, the vaginal discharge is normal vaginal discharge (physiological) such as clear, no odor, no itching and the amount is not excessive, whereas, if the fluid changes to a yellow color, smells, and is accompanied by itching, it is declared pathological vaginal discharge. (Susanty, 2021). Chlamydia trachomatis as one of the causes of vaginal discharge is a common occurrence in sexually active women. This is indicated by the highest number of 10-14% in the group under the age of 25 years (Rahayu et al., 2021).

World Health Organization (WHO) globally, it is estimated that there are 8%-10% of infertility cases in couples. Infertility is basically a disorder in the female reproductive system that causes pregnancy failure, even though active sexual intercourse for 12 months or more without using contraception (Cavallini & Beretta, 2015). Causes of infertility can occur due to female reproductive health problems, namely problems with ovulatory dysfunction, endometriosis, poor nutrition, hormonal disorders, to impaired sperm transport from the cervix to the fallopian tubes (Dhyani et al, 2020). Infertility data at the Timor-Leste National Hospital were obtained from records medical staff in 2020 to 2021 with a total of 57 people.

To perform CT bacterial examination using the Polymerase Chain Reaction (PCR) method on women with a first catch urine sample. Tissue culture as the gold standard has a specificity reaching 100% and sensitivity of 30-88% when performed by an expert. PCR examination has a higher sensitivity because it

is able to detect 1-10 elementary bodies and its specificity is comparable to culture. The sensitivity and specificity of the Polymerase chain reaction (PCR) method are 91.7% and 99.7%, so the nucleic acid amplification test has been approved by Food and Drug Administration (FDA) to replace the culture method as the gold standard for CT infection diagnostic tests (Wahyudi et al, 2019). Falk and his colleagues compared urine samples and endo-cervical samples to detect CT using the PCR method on infertility women from 171 women with CT infection with an endo-cervical sample sensitivity of 97.1% while a urine sample of 87.7% (Nadeak, 2019)

This study carried out at the National Hospital of Timor-Leste. From the data collected, it was found that so far there has been no definite diagnosis of CT infection, all cases found were only suspect and doctors immediately gave treatment. The application of urine PCR was carried out at the Timor-Leste National Hospital because there were no adequate laboratory facilities to carry out CT infection and infertility examinations. CT infection examination is only a urine PCR test. The description of the background above allows the researcher to analyze the relationship between vaginal discharge and Chlamydia Trachomatis in infertility by PCR examination of urine. The purpose of this study was to analyze the relationship between vaginal discharge and Chlamydia trachomatis (CT) in infertility by urine PCR examination at the Timor-Leste National Hospital.

2. Methods

Study design and setting

This study has a cross-sectional design and is an analytical observational study. The National Institute of Dili, Timor-Leste, issued this study an ethical clearance permit with the number 1168 MS-INS/GDE/VI/2022. Patients receiving therapy at the Timor-Leste National Hospital for infertility comprised the study's sample. The study was place from June–July 2022. The investigation was carried out at the

Timor-Leste National Laboratory (LABNAS) and the Guido Valadares National Hospital in Dili.

Sample inclusion criteria:

1. Patients with infertility who agree to participate in the study or act as respondents.
2. Infertile women aged 15-49 years

Sample exclusion criteria:

1. Laboratory samples do not meet the requirements
2. Contaminated samples

Sample size

The sampling method used in this study is Consecutive sampling. The following formula is used to get the total number of respondents or patients at the Timor-Leste National Hospital using the following calculation.

$$n = \left\{ \frac{Z\alpha + Z\beta}{\frac{1}{2} \ln \frac{(1+r)}{(1-r)}} \right\}^2 + 3$$

$$n = \left\{ \frac{1.96 + 0.84}{0.5 \ln \frac{(1+0.5)}{(1-0.5)}} \right\}^2 + 3$$

$$n = \left\{ \frac{2.8}{0.5 \ln(3)} \right\}^2 + 3$$

$$n = \left\{ \frac{2.8}{0.549} \right\}^2 + 3$$

$$n = \{5, 10\}^2 + 3$$

$$n = 26.01 + 3$$

$$n = 29.01 = 30$$

with n was the sum sample. $Z\alpha$ was standard derivative alpha (1.96), $Z\beta$ was standard derivative beta (0.84), and r was the correlation coefficient of previous research. The sample used in this study included up to 30 survivors of patients who had been treated for and identified as having Chlamydia trachomatis (CT)

infections at the National Hospital of Timor-Leste.

Data Collected

The patient's urine was collected and placed in a micro-centrifugation tube. PBS and proteinase are added during the process of preparing a urine sample. The cell lysis process was then started by combining the urine sample with GBS Buffer. Five minutes were spent incubating urine samples at 60°C. Adding ethanol, shaking, and combining it with the GD column were the steps to isolate the DNA. If the combination does not entirely pass through the GD Column membrane, the centrifugation duration should be increased following centrifugation. Discard the water collection tube, then transfer the GD Column to a new collection tube. The urine sample was centrifuged after being washed until the matrix column was dry. The GD column was then transferred to a clean micro-centrifugation tube, elution buffer was added, centrifuged, electrophoresed, and analyzed by PCR. Furthermore, bivariate and univariate data analysis were used in the data analysis process. Using the Chi-Square test with SPSS (Statistic Product Service Solution) software, it is possible to determine whether a relationship exists between two variables with a significant or 0.05 (5 percent) truth. If $\rho < 0.05$, then there is a relationship between vaginal discharge and Chlamydia trachomatis (CT), while $\rho > 0.05$, then there is no relationship (CT).

3. Results

The survey sample consisted of 30 respondents with the characteristics mentioned in Table 1 who had been treated and diagnosed with Chlamydia trachomatis (CT) infection at the Timor-Leste National Hospital.

Table 1. Characteristics of Respondents

Characteristic	n	%	Mean ± SD
Age (years)	30		31.17 ± 4.170
Length of marriage (years)	30		5.03 ± 2.371
Occupation			

Household	4	13.3
Nurse	2	6.7
Government Officer	14	46.7
Private	10	33.3
Education		
Junior High School	1	3.3
Senior High School	14	46.7
Diploma	1	3.3
Bachelor	14	46.7
Contraception		
Not used	30	100

Identification of vaginal discharge. Chlamydia trachomatis and infertility factors by PCR examination of urine and HSG results

According to the findings of the title of vaginal discharge in infertility (Table 2), 50% The 30 respondents who experienced vaginal discharge had the same results as those who did not experience vaginal discharge.

Table 2. Results of identification of vaginal discharge. CT. and infertility factors with urine PCR examination and HSG results.

Vaginal discharge	n	%
Yes	15	50
No	15	50
CT		
Positive	13	43.3
Negative	17	56.7
Infertility Vaginal		
Normal	2	6.7
Bilateral hydrosalpinx	4	13.3
Unilateral hydrosalpinx	4	13.3
Hyperplasia	2	6.7
Cyst	2	6.7
PCOS	3	10
Salpingitis	7	23.3
Endometriosis	4	13.3
Asthenozoospermia	1	3.3
Teratozoospermia	1	3.3

Relationship between vaginal discharge and Chlamydia trachomatis in infertility

Table 3 showed that from 30 respondents, infertile vaginal discharge was positive (53.3%)

and negative (46.7% While Infertile did not have vaginal discharge. It is positive (33.3%) and negative (66.7%).

Table 3. Results of the relationship between vaginal discharge and CT with urine PCR examination.

Vaginal discharge	PCR CT		p-value
	Positive	Negative	

Yes	8 (53.3%)	7 (46.7%)	0.462
No	5 (33.3%)	10 (66.7%)	

Chi-Square test results obtained p-value = 0.462 ($p > 0.05$) so that there is no relationship between vaginal infertility and urine CT PCR examination.

Of the 30 respondents, the relationship between infertility factors by HSG and urine CT was positive for salpingitis (38.5%) compared to negative Endometriosis (17.6%) from the others as shown in table .4

4. The relationship of infertility factors by HSG to Chlamydia trachomatis on infertility

Table .4 Relationship of Infertility Factors performed by HSG with Urine CT.

Infertility Factor	Urine CT PCR		p value
	Positive	Negative	
Normal	0 (0%)	2 (11.8%)	0.319
Bilateral hydrosalpinx	3 (23.1%)	1 (5.9%)	
Unilateral hydrosalpinx	2 (15.4%)	2 (11.8%)	
Hyperplasia	0 (0%)	2 (11.8%)	
Cyst	0 (0%)	2 (11.8%)	
PCOS	1 (7.7%)	2 (11.8%)	
Salpingitis	5 (38.5%)	2 (11.8%)	
Endometriosis	1 (7.7%)	3 (17.6%)	
Asthenozoospermia	0 (0%)	1 (5.9%)	
Teratozoospermia	1 (7.7%)	0 (0%)	

Fisher's Exact test results showed that there were no Infertility Factors performed by HSG with Urine CT p value > 0.05 .

More bilateral patent tubes on HSG examination were negative on CT urine, 82.4% compared to positive 53.8% in bilateral Non-patent tubes than others . Can be seen in table 5.

5. The relationship between HSG and Chlamydia trachomatis on PCR Urine

Table 5. Results of the relationship between HSG and Urine CT

HSG	Urine CT PCR		p value
	Positive	Negative	
Bilateral patent tube	0 (0.0%)	14 (82.4%)	0.001
Bilateral non-patent tube	7 (53.8%)	2 (11.8%)	
Unilateral non-patent tube	6 (46.2%)	1 (5.9%)	

Fisher's Exact test showed that there was a relationship between HSG and Urine CT PCR with $p < 0.05$ with a strong relationship strength ($C = 0.634$; $p > 0.001$).

July 2022. This study used data obtained directly of patients who come for treatment with infertility problems in House Sick National Timor Leste with examination urine polymerase chain reaction (PCR) and HSG at the Timor Leste National Hospital. Study this is study observational analytics

4. Discussion

This research was conducted from June -

Cross sectional with 30 sample respondent

Characteristics of Respondents

Respondents in this study had an average age of 31.17 with a standard deviation of 4.170. The standard deviation value which is smaller than the average value indicates that the age diversity of the respondents is small. This means that respondents have an age that is not much different from one another. In terms of length of marriage, it was found that the average length of marriage of respondents was 5.03 with a standard deviation of 2.371. The standard deviation value which is smaller than the average value indicates that the respondent's length of marriage has a small variation. The most occupations in the civil servant group (46.7%) and the highest education in the high school and undergraduate group (46.7%) and all respondents do not use contraception (100%).

In this study, age was not investigated in relation to Chlamydia trachomatis infection in infertility although in research conducted by (Kavanagh et al . 2013) found there is connection Among age with infection Chlamydia trachomatis on patient infertile and is factor risk occur tubal factor infertility (TFI) followed by Chlamydia trachomatis infection in women in Scotland. (Kavanagh et al . , 2013) Connection age with infection Chlamydia trachomatis often connected with behavior lots of sex partner by significant increase risk infection Chlamydia trachomatis . Woman infertility with infection Chlamydia trachomatis is estimated around 37% have partner sexual 3 person or more during 42 month. (Menon et al. 2016)

Identification of vaginal discharge, Chlamydia trachomatis and infertility factors on urine PCR examination and HSG results.

Of the 30 respondents who had the same results, those who had vaginal discharge and those who did not have vaginal discharge (50%) and the positive

Chlamydia trachomatis (43.3%) were smaller than the negative ones (56.7%) while the infertility factor was salpingitis (23.3%) more than others .

Results this research show whiteness not associated with Chlamydia trachomatis infection . Research result this fits with research done by (Geisler et al . 2015) that whiteness abnormal has no relationship with infection Chlamydia trachomatis (Geisler et al . , 2015) . However, according to study meta analysis get results which different that Symptoms of vaginal discharge can be used as an innovation of Chlamydia trachomatis infection which has a rate of achievement results which good with sensitivity around 91.68%, specificity 99.97% and positive predictive value (PPV) 99.93% (Zemouriet al . , 2016) , while when seen from trend percentage whiteness to Chlamydia trachomatis infection is more likely to occur on group woman infertile.

The results of other studies that whiteness more often found as symptomatic of urogenital infection in about 54 patients from 109 patients in total or about 50% (Geisler et al . , 2015). Other research whiteness could made diagnosis symptomatic around 26.5% infection vaginalis which caused by Chlamydia trachomatis (Wangnapi et al . , 2015) Study Sari in field report comparison 39 sample urine and endocervix on 39 patient which suspected cervicitis with method PCR and 9 samples (23%). (Sari, 2017) While Research Mittal in India reported the prevalence of genital Chlamydia trachomatis in women with method PCR use sample urine to 160 person womanobtained results positive Chlamydia trachomatis as much 15 person (9.38%). (Mitta et al . , 2010)

Hjelm 's study in Sweden compared urine samples, cervical and vaginal swabs in detecting Chlamydia trachomatis by the LCR method in women who went to a women's health clinic, the results of a positive urine sample for Chlamydia trachomatis by 39 samples (4%) Results

PCR positive Chlamydia trachomatis on preparation urine showing there is urethritis by because Chlamydia trachomatis . Cell epithelium on urethra is one of the tropisms primarily Chlamydia trachomatis . (Hjelm et al ., 2001)

Relationship of vaginal discharge to Chlamydia trachomatis in Infertility

Infertile vaginal discharge was positive (53.3%) and negative (46.7%). While Infertile was not vaginal discharge, positive (33.3%) and negative (66.7%). Based on the results of the analysis, it was found that there was no relationship between vaginal discharge and CT PCR results. This means that respondents who experience vaginal discharge or who do not experience vaginal discharge have nothing to do with Chlamydia trachomatis .

Many factors affect vaginal discharge in productive women. Lack of attitudes and knowledge in performing external genitalia hygiene care (external genitalia), as well as poor behavior trigger vaginal discharge (Azizah, 2015). Factors causing complaints in the vagina, mostly due to vaginal infections caused by germs, fungi, viruses and parasites (Azizah, 2015). However, the results of the Fisher's Exact test showed that there was a relationship between HSG and CT $p < 0.05$, with a strong relationship strength ($C = 0.634$; $p > 0.001$).

This study has no relationship between vaginal discharge and Chlamydia trachomatis on infertility However from the sample studied, it was found that Chlamydia trachomatis infection was more on woman infertile vaginal discharge compared to Infertile women do not have vaginal discharge. This result is suitable with Verweij's research that Chlamydia trachomatis antibodies are significantly frequently found in group woman infertile vaginal discharge which suffer TFI (Verweij et al ., 2015). The occurrence of pathogen interactions so that development infertility on infection Chlamydia trachomatis caused by infection ascending channel reproduction part on on a number of woman so that develop

happening pathology tube. Thing this proven with existence detection positive DNA Chlamydia trachomatis on endometrial, tube fallopian and ovary around 56% on woman with pregnancy ectopic or TFI. Besides it is also suspected that delayed hypersensitivity by cHSP60 antigen Chlamydia which could make response activity immunity during development condition pathology. (Menon et al ., 2015)

Tubal damage can vary from visible intra-tubal adhesions and hydrosalpinx to milder forms without visible tubal occlusion (Akande, 2007) Research this conclude existence risk TFI on patient with Chlamydia trachomatis infection (Dehghan Marvast et al ., 2016). Temporary research (Zhu et al . 2017) found an incidence rate of infection Chlamydia trachomatis which more tall on woman infertile caused by an anatomically increased risk of infection and a high risk of infectious infection sexual (STI) in women .(Zhu et al ., 2015).

Relationship of infertility factors by HSG to Chlamydia trachomatis urine .

Of the 30 respondents the relationship of infertility factors with positive PCR (38.5%) salpingitis compared to negative (17.6%) Endometriosis and more than others, none of these positive ones is normal. In this study, it was significantly lower in hydrosalpinx patients compared to salpingitis patients which is in line with previous studies (Kawwassk ., 2013). It has been shown that women with untreated hydrosalpinx are at a higher risk. but according to current opinion, most women with PID are not infected with chlamydia (Brunham et al ., 2015).

In a recent study, Chlamydia trachomatis was detected in only 15-30% of PID cases (Goller et al., 2016). The inflammatory response in the upper genital tract can damage the Fallopian tube tissue by reducing the number of ciliated mucosal cells and causing functional tubal damage (Shao et al., 2012). Such dysfunction may result in impaired ovum transport, lower odds of spontaneous pregnancy, It is also possible that C.

trachomatis impairs fertility by mechanisms other than damaging the fallopian tubes, such as causing chronic endometritis or post-infectious inflammation of the endometrium (Bouet et al ., 2016)

Relationship of HSG with Chlamydia Trachomatis in Urine PCR

Bilateral patent tubes on HSG examination were more negative on CT urine (82.4%) than positive (53.8%) on bilateral non-patent tubes and more than others. Many factors affect tubal patency. The requirements for a good tube are that it must be patent, free of adhesions, good fimbriae, good walls of the tube (villi/cillia/intralumen). HSG can only see tubal patency, whereas laparoscopy can see not only tubal patency, fimbriae, but also peritoneal factors (peritubal adhesions and endometriosis). Laparoscopy can not see the tubal intraluminal. Meanwhile, the tubal intraluminal can only be seen with special tools such as fertiloscopy and falloscopy. However, this examination is difficult to perform, requires special skills, and falloscopy is rarely performed because of the risk of tubal perforation (Fritz & Speroff, 2011).

Examination to determine abnormalities of tubal factors that are easy, inexpensive and non-invasive is the Hysterosalpingography (HSG) examination. HSG can only see tubal patency, while other tubal anatomy and peritoneal factors cannot be seen. In all infertile patients, HSG has an error of 60% to detect tubal patency, meaning that if the HSG is obstructed, there is a high probability (60%) that the tube is open on laparoscopy. While the standard infertility examination to determine tubal and peritoneal factors is to use laparoscopy. In addition to tubal patency, abnormalities of peritoneal factors can also be identified (Fritz & Speroff, 2011).

There is no significant relationship between Chlamydia infection trachomatis to infertility in this study and can also due to multiple factors, who does not checked on research These include blood tests for IgG and IgM and translocated actin recruiting phosphor

protein (TARP) synthesized during CT infection and can be show pathogenic which critical.(Mehlitz and Rudel, 2013) . The results of this study are different from the research conducted by (Malik et al . 2012) .

IN Aligarh on 130 woman infertile, where found 31 case infected Chlamydia trachomatis and there is significant relationship Among infection Chlamydia trachomatis with infertility (Malik et al ., 2012). This difference in results is due to the number of case samples in Malik's study . more that is as much as 130sample, the number of samples of the control group (30 samples) is not equal to number of case group samples (100 samples), and examination method Chlamydia trachomatis was carried out by 2 methods, namely culture and monoclonal antibody test . While in this study, the sample totaling 30 samples and method inspection Chlamydia trachomatis with use 1 method inspection that is technique Urine PCR examination with Xpert CT/NG. In addition, the clinical manifestations of this study were found increasing trend of Chlamydia trachomatis infection in women infertile vaginal discharge but no could explained by statistics Thing this possible caused by infertility character multifactorial where infection Chlamydia trachomatis only wrong one factor the cause.

Limitations of the researcher

No comparison check Chlamydia trachomatis which are related with infertility such as IgG and IgM blood tests, vaginal swabs, endocervical swabs and others to detect vaginal discharge with Chlamydia trachomatis in infertility

Conclusion

1. Results Identification of vaginal discharge in infertility which has the same results between vaginal discharge and no vaginal discharge and Chlamydia trachomatis in infertility with a negative urine PCR

- examination is greater than the positive result.
2. The results of the identification of infertility factors with salpingitis were more positive than the others.
 3. Vaginal discharge not associated with Chlamydia trachomatis in infertility by urine PCR examination at the National Hospital of Timor Leste.
 4. Factors of Infertility not associated with Chlamydia trachomatis by urine PCR examination at the Timor Leste National Hospital
 5. There is a relationship between HSG and PCR for Chlamydia trachomatis at the Timor Leste National Hospital.

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