ORIGINAL ARTICLE

Prevalence of *Chlamydia trachomatis* among Subfertile Couples of LPPKN Subfertility Clinic

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ABSTRACT

Introduction: *Chlamydia trachomatis* is the most prevalent sexually transmitted infection world wide caused by the bacterial agent named *Chlamydia trachomatis*. Long term chlamydial infection has a negative impact on the female reproductive system and often leads to infertility. We determined the prevalence of *Chlamydia trachomatis* and its associated factors among subfertile couples attending the Lembaga Penduduk dan Pembangunan Keluarga Negara (LPPKN) Subfertility Clinic. **Method:** Blood samples were collected from 95 couples (190 respondents) for *Chlamydia trachomatis* IgG antibody testing. The Enzyme-Linked Immunosorbent Assay (ELISA) technique was used to detect outer membrane protein complexes of *Chlamydia trachomatis* in the assay. **Results:** *Chlamydia trachomatis* IgG antibody seropositivity was detected in 14 husbands (14.7%) and 17 wives (17.9%). The prevalence of *Chlamydia trachomatis* among these couples was 22.1%. Our findings found no significant association between *Chlamydia trachomatis* and its associated factors, but the prevalence rate among the studied population is of concern. **Conclusion:** The screening of *Chlamydia trachomatis* among couples is recommended for integration in the subfertility investigation procedure. It is crucial, particularly for women with tubal factor infertility (TFI).

Keywords: *Chlamydia, Chlamydia trachomatis,* Infertility, Subfertile couples, Prevalence

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INTRODUCTION

Chlamydia trachomatis is the bacterial agent responsible for chlamydia, a sexually transmitted infection (STIs). It is a unique gram-negative bacterium that is biphasic (i.e., having two stages) known as the resting phase (elementary body, EB) and the replication phase (reticulate body, RB). This characteristic enhances its survival in the reproductive tract (1). Chlamydia is less prominent compared to human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), gonorrhoea or syphilis, especially in Malaysia, even though it is reported as the most common sexually transmitted disease (STD) worldwide. Global estimates of chlamydia among women were between 2.4% – 6.9% (2). As this disease is asymptomatic, carriers are often unaware of the infection, causing delays in treatment or rendering the infection untreatable.

Long-term chlamydial infection significantly increases the risk of infertility, especially in women. Although is not life-threatening, it can inflict a psychological-emotional burden on a family. Nowadays, many developed countries are facing dwindling fertility rates among young couples. Malaysia has experienced a decline in total fertility rate (TFR - a number of children born per woman) from 3.6 children per woman in 1985 to 3.0 children per woman in 2000 and has reduced further to 2.5 children per woman in 2005 and 2.3 children per woman in 2008 (3). Centres for Disease Control and Prevention (CDC) has stated that infertility could be caused by various factors, such as genetic abnormalities, infectious agents and environmental factors (4). There

have been several findings that associate *Chlamydia trachomatis* infection with infertility, particularly in the tubal factor infertility (TFI) and pelvic inflammatory disease (PID) (5, 6). A few studies on chlamydia have been carried out in Malaysia since 1990. At the time, sexually transmitted infections were frequently associated with low social statuses, such as that of sex workers and drugs addicts. Ngeow et al. (1997) have shown that the prevalence of positive chlamydia antibody tested among a total of 794 infants, adolescents and adults, including sex workers, was 45.5% (7). Whereas, the incidence of asymptomatic Chlamydia infection among infertile women was reported to be 33.3% (8). Additionally, Ravindran et al. (1998) found that the prevalence rate of Chlamydia infection among pelvic inflammatory disease (PID) patients in Seremban Hospital were 22.7% (9). A retrospective study of patients attending Genito-Urinary Medicine (GUM) Clinic, General Hospital Kuala Lumpur, revealed 23.0% males and 17.5% females were chlamydia positive (10).

Information on the prevalence of *Chlamydia trachomatis* infection among subfertile couples in Malaysia is limited. Firstly, this could be related to difficulties in conducting the study in an infertility centre; secondly, data related to infertility issues can only be collected if the patient comes forward and seeks treatment; and thirdly, data can only be reported if the chlamydia tests are offered at the fertility centre. Since infertility is known as the long-term effect of *Chlamydia trachomatis* infection, the detection of IgG antibody is an ideal approach, where it is an indicator of past infection(s) and remains detectable for years post-infection (11). The blood sample can be used for screening purposes as it is easily obtained through venipuncture compared to other invasive methods such as cervical swab or urethral swab. We determined the prevalence of *Chlamydia trachomatis* infection among infertile couples at the Lembaga Penduduk dan Pembangunan Keluarga Negara (LPPKN) Subfertility Clinic and associated them with the sociodemographic of the respondents.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Lembaga Penduduk dan Pembangunan Keluarga Negara (LPPKN) Subfertility Clinic located in the heart of Kuala Lumpur. The recruitment was between June 2018 to October 2018. Married couples (husband and wife) that were registered as patients at the LPPKN Subfertility Clinic were invited to participate in the study. Couples were approached and screened for the inclusion and exclusion criteria. The inclusion criteria were: those who were trying to get pregnant with at least one (1) year of regular and unprotected sexual intercourse (sex without condom or pills), clients with primary and secondary infertility, compulsory participation of both partners (husband and wife) in the study, no antibiotics treatment either via the oral route, injection or vaginal suppository for at least one month before blood sampling and couples who consented for enrolment. Those respondents who could not understand Bahasa Malaysia and English and who had a history of non-communicable medical conditions known to affect fertility (e.g.; cancer, thyroid problem, sickle cell disease, etc) were excluded from the study.

Sociodemographic characteristics (age, ethnicity, education level, occupation and income), duration of the marriage, diagnosis of infertility and infertility factors were extracted from the medical records and recorded into a proforma.

Specimen Collection and Laboratory Test

Blood (5 ml) was taken through a routine venipuncture procedure and collected into a sterile plain tube. This procedure was conducted by trained health care staff. The blood sample was then centrifuged at 1,000 rpm for 10 minutes to separate the serum which was subsequently stored at -20°C for the ELISA test.

The blood samples were used to detect serum *Chlamydia trachomatis* (CT) outer membrane protein complexes IgG antibodies. The enzyme-linked immunosorbent assay (ELISA) technique was employed out according to the manufacturer's instructions in Vircell Microbiologist (Granada, Spain) with a sensitivity of 96% and specificity of 98%. All reagents were placed in room temperature for approximately 1 hour before use and agitated well. Cut-off control 1 and 2, negative control, positive control, and serum diluents were included in each plate apart from clients' samples. Briefly, a mixture of 100 µl of diluents with 5 µl of cut-off control 1 and 2, negative control, positive control and samples, respectively, were added to each corresponding well. The plate was then agitated for 2 minutes to achieve a homogenous mixture. Subsequently, the plate was covered with a sealing sheet and incubated at 37°C for 45 minutes. After that, the plate was washed five times with washing solution. Then, 100 µl of IgG conjugate solution was added. The plate was covered and incubated for 30 minutes at 37°C. Next, all liquid was aspirated, and the plate was washed five times with washing solution, followed by, 100 µl of substrate solution and incubated at room temperature for 20 minutes in the dark. Immediately, 50 µl of stopping solution was added. The plate was read at 450/620 nm with a spectrophotometer within one hour of the stopping solution. The whole process was conducted by using ELx50TM Microplate Strip Washer and ELx800TM Absorbance Microplate Reader (Vermont, USA).

To ensure the reliability and validity of the test, ELISA instruments and pipette (washer and reader) were calibrated before actual testing. A few performance verifications were employed to ensure reliable results were produced, for example, each sample was loaded in duplicates to get the mean value; two cut-off levels

(1 and 2), positive and negative controls were loaded concurrently in every test batch; a new microplate that was clean and free from any dust or bottom scratches was utilized to ensure minimal reading error; flat-bottomed wells for best optical clear reading and an empty microplate reading as baseline control. Dual-wavelength filters readings were used, to measure each well with 450 nm and 620 nm of wavelength (in the ELISA reader) to get the variation in density and average measurements within the acceptable limits.

Chlamydia trachomatis was defined as positive with the presence of *Chlamydia trachomatis* IgG antibody (CT IgG) in the serum sample. Couples were identified as CT-positive if either the husband, the wife (serodiscordant), or both (seroconcordant), were found seropositive of *Chlamydia trachomatis* (12, 13). Meanwhile, couples were identified as CT-negative if both husband and wife were found seronegative of *Chlamydia trachomatis*.

Statistical Analysis

Based on the previous study using a similar model for sample size calculation, an estimated minimum sample size of 230 respondents was required to have 95% confidence interval with 5% margin of error (14, 15) All data was encoded and analyzed using the Statistical Package for Social Sciences (SPSS) Version 23, IBM, USA. A descriptive analysis (frequencies and percentages tables) was performed for nominal and categorical data. The categorical data were not normally distributed, therefore, a non-parametric analysis (chi-square and Fisher's exact tests) was used. The results were assumed to be statistically significant when $p < 0.05$.

Ethical Approval

This study was approved by the Ethics Committee for Research Involving Human Subjects Universiti Putra Malaysia (FPSK-P153) 2017, and the informed consent forms (ICFs) were given to respondents to ensure the patients' rights, confidentiality, voluntary participation, and sample management were taken into consideration.

RESULTS

Demographic characteristics

The response rate was 84.8%. A total of 112 couples (224 respondents) agreed to participate in the study. However, only 99 husbands and 95 wives gave blood samples. Thus, only 95 analysis by couples (190 respondents) were conducted. The details of the number of specimens and laboratory tests performed are attached (Figure 1).

Socio-Demographic Profile of Respondents

In this work, most of the respondents were aged between 25–34 years old (75.3%) and only 0.5% were below 25 years old. The study samples were dominated by the Malay ethnicity (92.1%), while the remaining 7.9% were non-Malays. Meanwhile, 51.6% of respondents had a tertiary level education (university level), 32.1% were college graduates and 16.3% were secondary school leavers. The majority of them (73.7%) were working in the private sector, while 21.0% were from the government sector and the remaining 5.3% were housewives. Nearly half of the total respondents (48.4%) had an income ranging between RM2,000–RM3,999, 21.0% with income ranging between RM4,000–RM5,999, and 17.9% had an income below RM2,000. However, among the 5.3% housewives, some were without an income (Table I).

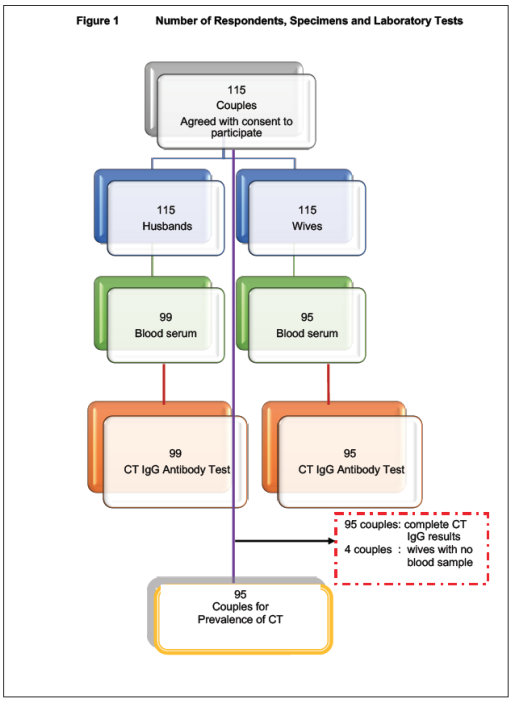

Figure 1 : Number of Respondents, Specimens anf Laboratory tests

Duration of Marriage, Diagnosis of Infertility and Infertility Factor of Respondents

Half of the respondents were couples who have been married for 3–7 years (51.6%), less than 3 years were 32.6%, and 13.7% were married for 8–12 years The majority of them had primary infertility (73.7%) and 26.3% had secondary infertility. The female factor was reported to be most dominant (37.9%), followed by the unexplained factor (24.2%), and then the male factor (16.8%) (Table II).

Prevalence of *Chlamydia trachomatis*

Prevalence of *Chlamydia trachomatis* among subfertile couples in the LPPKN Subfertility Clinic was 22.1%. Meanwhile, prevalence among husbands and wives were 14.7% and 17.9%, respectively (Table III).

No significant association was found between CT status and age, ethnicity, education level, occupation and income among husbands ($p > 0.05$) (Table IV). Although age was not statistically significant, the results indicated that positive CT was found to be higher (17.2%, 5/29) among male respondents aged 35 years

and older compared to those who are 34 years and younger (12.9%, 9/70). The prevalence of positive CT was highest among husbands with secondary school education (21.7%, 5/23), compared to those who received college (17.1%, 6/35) or university education (7.3%, 3/41). Those husbands who were positive CT, the majority (15.4%, 4/26) are civil servants and husbands with income less than RM 2,000 (23.1%, 3/13).

There were also no statistically significant associations between CT status among the wives in relation to age, ethnicity, education level, occupation and income ($p > 0.05$) (Table V). For positive CT female respondents (Wives), the majority were 34 years old and younger (21.1%, 16/76) compared to those more than 34 years old (5.3%, 1/19). Like the husband's result, positive CT was relatively higher in secondary school leavers (27.3%, 3/11) followed by the college (23.1%, 6/26) and university graduates (13.8%, 8/58). In terms of occupational status, the highest were reported among civil servants (20.0%, 3/15) and housewives (20.0%, 2/10) with income between RM 2,000- RM 3,999 (23.3%, 10/43).

Table I : Sociodemographic Characteristics of the Respondents (CT IgG Antibody) (N = 190)

SD INFORMATION	HUSBAND (n = 95)		WIFE (n = 95)		TOTAL (N = 190)	
	n	%	n	%	N	%
Age						
< 25 years old	-	-	1	1.1	1	0.5
25–34 years old	68	71.6	75	78.9	143	75.3
35–44 years old	24	25.3	18	18.9	42	22.1
> 44 years old	3	3.2	1	1.1	4	2.1
Ethnicity						
Malay	87	91.6	88	92.6	175	92.1
Non-Malay	8	8.4	7	7.4	15	7.9
Education Level						
Secondary School	20	21.1	11	11.6	31	16.3
College	35	36.8	26	27.4	61	32.1
University	40	42.1	58	61.0	98	51.6
Occupation						
Government Sector	25	26.3	15	15.8	40	21.0
Private Sector	70	73.7	70	73.7	140	73.7
Housewife	-	-	10	10.5	10	5.3
Income						
< RM2,000	11	11.6	23	24.2	34	17.9
RM2,000–RM3,999	49	51.6	43	45.3	92	48.4
RM4,000–RM5,999	27	28.4	13	13.7	40	21.0
RM6,000–RM7,999	6	6.3	4	4.2	10	5.3
> RM8,000	2	2.1	2	2.1	4	2.1
*No Income	-	-	10	10.5	10	5.3

*No income refers to the 10.5% housewives

Table II : Duration of Marriage, Diagnosis of Infertility and Infertility Factor of Couples (CT IgG Antibody) (N= 95)

	Couple (N = 95)	
Duration of Marriage	n	%
< 3 years	31	32.6
3–7 years	49	51.6
8–12 years	13	13.7
> 12 years	2	2.1
Diagnosis of Infertility		
Primary	70	73.7
Secondary	25	26.3
Infertility Factor		
Male	16	16.8
Female	36	37.9
Combined (Male and Female)	13	13.7
Unexplained (Idiopathic)	23	24.2
Wife Discontinued	7	7.4

Table III : Prevalence of *Chlamydia trachomatis* Among Subfertile Couples (N = 95)

	HUSBAND CT	WIFE CT	COUPLE'S CT	FREQUENCY (N = 95)	PERCENTAGE (%)
	Positive	Positive	Positive	10	10.5
	Positive	Negative	Positive	4	4.2
	Negative	Positive	Positive	7	7.4
	Negative	Negative	Negative	74	77.9
Prevalence of CT among:	14.7%	17.9%	22.1%		

Table IV : Association between *Chlamydia trachomatis* (CT) Status and Sociodemographic Characteristic of the Male Respondents (Husbands) (N = 99)

Variables	Husband CT Status				p-value
	Negative CT (-) n = 85		Positive CT (+) n = 14		
	n	%	n	%	
Age					0.569
≤ 34 years old	61	87.1	9	12.9	
≥ 35 years old	24	82.8	5	17.2	
Ethnicity					0.231
Malay	77	84.6	14	15.4	
Non-Malay	8	100.0	0	0.0	
Education Level					0.232
Secondary School	18	78.3	5	21.7	
College	29	82.9	6	17.1	
University	38	92.7	3	7.3	
Occupation					0.832
Government Sector	22	84.6	4	15.4	
Private Sector	63	86.3	10	13.7	
Income					0.397
< RM2,000	10	76.9	3	23.1	
RM2,000–RM3,999	43	84.3	8	15.7	
> RM4,000	32	91.4	3	8.6	

Table V : Association between *Chlamydia trachomatis* (CT) Status and Sociodemographic Characteristic of the Female Respondents (Wives) (N = 95)

Variables	Wife CT Status				p-value
	Negative CT (-) n = 78		Positive CT (+) n = 17		
	n	%	n	%	
Age					0.108
≤ 34 years old	60	78.9	16	21.1	
≥ 35 years old	18	94.7	1	5.3	
Ethnic					0.796
Malay	72	81.8	16	18.2	
Non-Malay	6	85.7	1	14.3	
Education Level					0.407
Secondary School	8	72.7	3	27.3	
College	20	76.9	6	23.1	
University	50	86.2	8	13.8	
Occupation					0.950
Government Sector	12	80.0	3	20.0	
Private Sector	58	82.9	12	17.1	
Housewife	8	80.0	2	20.0	
Income					0.234
< RM2,000	27	81.8	6	18.2	
RM2,000–RM3,999	33	76.7	10	23.3	
> RM4,000	18	94.7	1	5.3	

Table VI : Association of *Chlamydia trachomatis* (CT) and Duration of Marriage, Diagnosis of Infertility and Infertility Factor among Infertile Couples (N = 95)

Couple's SD Information	CT				p-value
	Negative (-) n = 74		Positive (+) n = 21		
	n	%	n	%	
Duration of Marriage					0.441
< 3 years	24	77.4	7	22.6	
3–7 years	36	73.5	13	26.5	
8–12 years	12	92.3	1	7.7	
> 12 years	2	100.0	0	0.0	
Diagnosis of Infertility					0.165
Primary	57	81.4	13	18.6	
Secondary	17	68.0	8	32.0	
Infertility Factor					0.715
Male or Female Factor	41	78.8	11	21.2	
Combined (Male & Female)	9	69.2	4	30.8	
Unexplained (Idiopathic) and Wife Discontinued	24	80.0	6	20.0	

* Wife discontinued means that the husband is normal, while the wife has no or unknown result

No significant association was found between the CT status of couples in relation to the duration of the marriage, diagnosis of infertility, and infertility factor ($p > 0.05$) (Table VI). Majority of the couples who tested positive for CT were those married between 3–7 years (26.5%, 13/49) and those less than 3 years (22.6%, 7/31). In terms of diagnosis of infertility, the majority were secondary (32.0%, 8/25) followed by primary infertility (18.6%, 13/70). The prevalence was higher among couples with a combination of male and female factors (30.8%, 4/13), then either male or female factors (21.2%, 11/52) and subsequently unexplained and wife discontinued factors (20.0%, 6/30). There was no significant association between the wives' CT status and infertility factor ($p = 0.715$).

DISCUSSION

Recently, the Ministry of Health Malaysia has released the latest HIV/AIDS country report documenting that the spread of HIV/AIDS has shifted from drug injection to sexual transmission by heterosexuals and homosexuals (16). The same route could also transmit *Chlamydia trachomatis* and cause chlamydia.

Chlamydia trachomatis is the most prevalent urogenital infection (17). Ascending infection from female lower genital tract upwards may lead to cervicitis, PID, and TFI which could impair the ability to conceive. Chlamydia is a serious health threat as it could also facilitate the HIV/AIDS virus to cross the border membrane of the epithelial cells and cause co-infections (18).

Conducting a study which involves the voluntary participation of married couples is a challenging task. Furthermore, there is limited data on *Chlamydia trachomatis*, specifically among married couples, since many studies were carried out either among women only or men only. Off the 95 subfertile couples tested in this study, the prevalence rate of CT was indicated to be 22.1% (21/95). This is lower than the prevalence reported in North-Western Nigeria (58.3%), however, it is higher than that documented in Iran and China which is 18.0% and 3.15% respectively (19 - 21). The high prevalence rate of CT among the participants in the current study was probably due to unprotected (i.e., without condom) vaginal intercourse since subfertile couples are known to be actively trying to conceive. Condom use is effective in preventing the transmission of Chlamydia and other STI between sexual partners (22). However, the present studied population was among those who came to subfertility clinics to seek fertility treatment and were therefore unaware of their CT status, thus, carrying with them the bacterial agents without receiving any proper treatment.

The present study also showed a higher prevalence rate of CT among wives 17.9% (17/95) compared to

husbands 14.7% (14/95). A similar study was investigated by Corbeto et al. (2014), who reported a higher prevalence rate of CT among women (6.6%) than among men (4.6%) (1). Similarly, Rawre et al. (2016) suggested a prevalence rate of positive CT of 10.0% among husbands and 15.7% among wives (23). The unequal rate of CT prevalence rate between husbands and wives could indicate that women are more susceptible to CT infection compared to men as urination may flush out the bacterium from the urethra in the male urogenital system while the female reproductive system acts as a reservoir of penetration and ejaculation from men (24). In addition, the nature of hormonal factors might play a role, since the estrogen has an immuno-enhancing effect in women whereas, the testosterone is immunosuppressive in men (25). This could explain the higher prevalence rate of CT IgG reported among the wives in our studied population as seen in the previous study as well as in other countries (14, 26 -27).

The current study focuses on the infertile couple, but it is also worthwhile to compare this with the reported data on fertile women. Odusolu et al. (2016) reported the prevalence rate of CT was found to be higher among infertile (38.6%) compared to pregnant women (22.8%) in Nigeria (28) and Menon et al. (2016) revealed a higher prevalence rate of CT among Samoan subfertile women (50.0%) (29). A higher CT IgG antibodies titre among women was found to be correlated with tubal factor destruction. The asymptomatic CT infection causes an inflammatory effect in the female reproductive tract causing a tubal blockage, even though it has been treated with an antibiotic (30, 31). Reinfection by the untreated husband may lead to a more serious impact and the disease may also cause embryo damage, thus, increasing the female factor in infertility (32).

Although more studies on the prevalence of CT among women have been reported by scholars, there were some studies conducted on male fertility. Noruziyan et al. (2013) reported a higher prevalence of CT IgG among infertile men (4.3%) compared to fertile men (3.2%) (33). They also observed that CT reduced the number of normal spermatozoa and its volume in seminal fluid, thus lowering the quality of sperm itself. Additionally, the spermatozoa are not protected from the negative impact of CT infection even after the 'sperm washing' procedure (34). These studies showed that chlamydia could have disturbed the fertility factors not only in women and men in vivo but also in vitro by sample processing in the laboratory.

Based on earlier studies, few factors have been reported to be associated with CT infection. In this study, the independent variables were age, ethnicity, education level, occupation, and income. Aside from those, marital status such as duration of the marriage, diagnosis of infertility, and infertility factor were also included. However, the association of such variables with CT infection were found to not be significant. The present study has reported the prevalence rate of CT to be higher among husbands aged 35 years and older (17.2%, 5/29) compared to wives aged 34 years and younger (21.1%, 16/76). The two groups of the age range (husbands and wives) are considered to be the age of maturity since the respondents have already been married for 3–7 years (26.5%, 13/49). This is different from the respondents in other studies which focus on younger ones who are single.

In terms of ethnicity, the studied couples were predominantly Malay (92.1%), therefore, the unbalance or limited proportion of ethnicity samples could not describe the difference of CT prevalence among various ethnic groups (35). Although CT infection was indicated to be higher among adolescents, the effect of ethnicity was still unclear, therefore, socioeconomic status or income has to be taken into account (35). In consideration of the participant's socioeconomic status, we hypothesized that a higher prevalence rate of CT among women respondents could be attributed to extramarital affairs in such cases as in a 'commuter marriage', where many couples have to live apart from their spouse due to work demands leading to infidelity with casual sex partners (other than their spouse) or with commercial sex workers (36). LPPKN, in their latest Fifth Malaysian Population and Family (MPFS-5) survey, reported that 'cheating' (infidelity) was ranked as the second-highest cause of divorce among men and women respondents (37).

We also determined that the prevalence rate of CT was higher among male (21.7%, 5/23), and female (27.3%, 3/11) respondents with secondary school education background, and among male civil servants (15.4%, 4/26) with income less than RM 2,000 (23.1%, 3/13). This could be because the fertility services offered by LPPKN is affordable among low- and moderate-income household couples compared those offered in private centres. Besides, LPPKN, as a semi-government agency under the Ministry of Women, Family and Community Development of Malaysia provides a special grant for civil servants who are suffering from primary subfertility to undergo subfertility treatment. Another interesting finding was that majority of CT positive were found among housewives (20.0%) and women civil servants (20.0%). This scenario might have explained why the prevalence of CT was reported higher among female compared to the male respondent. Our findings were consistent with the studies of Yeow et al. (2016) and Abdulrahman et al. (2016), the former showing an insignificant association among age, ethnicity and diagnosis of infertility (14), while the latter showing an insignificant association between age and diagnosis of infertility as well (38) respectively. In contrast, Odusolu et al. (2016) indicated a significant difference between

age and CT infection among infertile women in Calabar, Nigeria (28).

In this study, the majority of positive CT couples were among those who have been married for 3 to 7 years (26.5%, 13/49), have been diagnosed with secondary infertility (32.0%, 8/25) and with combined infertility factor (male and female factors) (30.8%, 4/13). Even though we found no significant association between these factors and CT infection, the findings indicate that CT positive was found higher among couples with longer duration of the marriage and could be the long-term impact of a past infection that occurred during their first exposure and was left untreated. The infection could be prolonged or be acquired through re-infection from the untreated partners. Our result was supported by the study of Smolak et al. (2019) in their large-scale population study among 20 countries in the Middle East and North Africa where the CT prevalence was recorded high among infertile patients (39). In contrary, Singh et al. (2016) reported a significant association between CT and infertility factor among infertile women in India (40).

Even though chlamydia could be treated by Azithromycin and Doxycycline with a single or 7-day dosage (41), repeated infections from a sexual partner could lead to CT drug-resistant strains (42). At present, there is no specific vaccine geared toward preventing CT (31). However, there was evidence that treating positive CT women reduced the risk of getting the post-abortion (43). Therefore, the good practice of healthy sexual behaviour and social lifestyle are the best preventions of social diseases not only for chlamydia but also for other sexually transmitted infections.

In reality, many people might have not heard or known chlamydia regardless of their social status or educational background. This situation could be attributed to the lack of knowledge and public awareness of this silent disease. We encourage couples to come forward voluntarily for CT testing and STI screening (for secondary prevention). We suggest that the authorities include chlamydia as a notifiable infectious disease in Malaysia following the footsteps of countries like Singapore, Brunei, United Kingdom, Australia, and other developed countries for national data collection and stored as references which are essential for researchers, particularly in the *Chlamydia trachomatis* field. An effective program on its prevention and on forecasting its impact among our adolescents and young adult population could also be made.

We highly recommend that the chlamydia antibody testing be integrated into subfertility investigation as a routine procedure. This is a necessary effort that needs to be taken for early detection of *Chlamydia trachomatis* infection, in hopes that the patients be treated and not infect their partners. Thus, the quality of subfertility treatment specifically on the assisted reproductive technology (ART) outcome will be improved, although the long-term impacts of this silent disease are still being studied.

We encountered several challenges throughout the study which includes some problems in collecting blood samples of both husband and wife. For example, azoospermia cases (no spermatozoa seen in seminal fluid) in male respondents or a spontaneous pregnancy has led to the case being dropped. Some patients went to other facilities to seek for the second opinion and some discontinued their participation due to other various reasons such as busy schedules, financial problems, and participants getting divorced. These factors reduced the number of potential respondents. Thus, the main limitation of this study was the small number of sample size. Since the present study was carried out on the infertile couple, voluntary participation by both (husband and wife) with complete clinical samples (blood) has decreased the number of couples to be analyzed.

CONCLUSION

In conclusion, the prevalence of *Chlamydia trachomatis* was quite high among infertile couples and this could be presumed as one of the causes of infertility. The findings of this study are expected to be evidenced-based of *Chlamydia trachomatis* infection among infertile couples and it would be valuable, especially for fertility centres, for effective diagnostic management on subfertility cases.

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