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Original Article

Is genital tract infection related to tubal diseases in infertile Vietnamese women?

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Abstract

Introduction: The goal of this study was to identify the profile of genital tract infections and their relationship with clinical and demographic parameters as well as tubal diseases among infertile women in Vietnam.

Methodology: In this cross-sectional descriptive study, we enrolled 597 women undergoing infertility treatment at the Center for Reproductive Endocrinology & Infertility, Hue University Hospital, Vietnam. All of the study participants were interviewed and examined by a gynecologist. Consecutive tests were then conducted including direct microscopy examination (wet mount and Gram stain), vaginal culture, polymerase chain reaction (PCR) for chlamydia diagnosis from a cervical canal swab, and a blood test for syphilis detection. A hysterosalpingogram (HSG) was carried out to examine the uterine cavity and Fallopian tubes.

Results: A gynecologic infection was diagnosed in 43.4% (259/597) of the infertile women. Bacterial vaginosis was the most common condition at 19.6% of the cases. *Candida* spp., *Chlamydia trachomatis*, and *Trichomonas vaginalis* infections accounted for 17.4%, 3.7%, and 0.3%, respectively. Normal HSG results accounted for 87.4% of the women while 5.5% had 2-sided tubal occlusions, 5.4% had 1-sided tubal occlusions, 1.0% had 1-sided hydrosalpinx, and 0.7% had 2-sided hydrosalpinx. There was no significant association between tubal diseases and current infections; however, aerobic vaginitis increased the risk of tubal diseases by 2.4 times.

Conclusions: A marked proportion of infertile Vietnamese women have genital tract infections that can significantly influence their reproductive function and performance. These infections should be routinely screened and treated properly to prevent their consequences, such as infertility, which is especially important in developing countries.

Key words: genital tract infection; infertility; tubal diseases; Vietnam.

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Introduction

Infertility accounts for approximately 9-15% of reproductive-aged women worldwide, leading to considerable costs of treatment and long-term psychological stress [1]. The burden of infertility is inordinately higher among women in developing countries; in some of regions of South and Central Asia, sub-Saharan and Northern Africa, the Middle East, and Eastern Europe, the infertility rate can reach up to 30% among reproductive-aged women [2]. Female infertility can be related to various causes, including ovarian dysfunction, acquired gynecologic tumors, or genital tract infections [3].

Bacterial vaginosis (BV) is the most common cause of vaginal discharge and is strongly associated with reproductive failure, notably late fetal loss [4]. *Candida*

albicans is a common commensal organism of the female genital tract but may occasionally cause diseases ranging from mild forms of vaginitis and cervicitis to serious illnesses [5]. *Trichomonas vaginalis* is a common vaginal parasite with worldwide distribution but is more common among women and individuals of a lower social status. Although trichomoniasis is associated with mild vaginal and cervical damage, it seems incapable of producing cervical factor infertility [5]. Concomitantly, several sexually transmitted diseases (STDs) such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma genitalium* infections can damage the columnar epithelium of the endocervix and spread to the endometrium and Fallopian tubes, contributing to pelvic inflammatory disease (PID) [6]. PID can permanently damage the reproductive system and is substantially associated with infertility [7]. Although the prevalence of gonorrhea infection has decreased, that of *Chlamydia trachomatis* infection remains elevated and is the main cause of tubal infertility, chronic pelvic pain, and ectopic pregnancy [8]. Moreover, in many cases, the signs and symptoms of higher genital tract infection are not notable and specific, which is why many patients remain undiagnosed.

The prevalence of genital tract infections (GTIs) is quite high in Vietnam, especially those that cause infertility [9–11]. Additionally, given the limited resources of developing countries, the treatment opportunities for infertile couples remain challenging. Therefore, the aim of this study was to identify the profiles of GTIs and their relationship with the clinical and demographic parameters and tubal diseases among infertile Vietnamese women.

Methodology

Patient population

Women in infertile couples undergoing infertility treatment at the Center for Reproductive Endocrinology & Infertility, Hue University Hospital, Vietnam, from June 2016 to June 2017 were recruited into this study. Exclusion criteria included menorrhagia, treatment for genital tract infection with systematic or local antibiotics within 4 weeks before inclusion, and unwillingness to be enrolled into the study population.

The sample size was calculated for the rate estimate investigation:

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

With the prevalence p = 44% [12], Δ = 0.02, α = 0.05, and $Z_{\frac{\alpha}{2}} = 1.96$, the minimum simple size was estimated to be 379 women. A total of 597 women were recruited for the study population during the recruitment period.

Ethical considerations

This study was approved by the Institutional Ethics Committee of Hue University of Medicine and Pharmacy, Vietnam. All of the study participants accepted and signed a consent form.

Study design, clinical investigations, and sample collection

This was a cross-sectional descriptive study. All of the women were interviewed according to a prepared protocol, collecting information about the demographic data, history of obstetrics and gynecology, history of genital tract infections, and previous management of GTIs and infertility.

Each subject was measured for height and weight. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters. Based on the Asian-specific classification for BMI status, BMI values were categorized as underweight (< 18.5 kg/m^2), normal (18.5-22.9 kg/m^2), overweight (23.0-24.9 kg/m^2), and obese (≥ 25 kg/m^2) [13]. Oligomenorrhea was defined as having fewer than 8 menstrual cycles per year, the absence of 3-6 consecutive menstrual cycles per year, or intermenstrual intervals ≥ 35 days.

A standardized physical and pelvic examination was carried out. One vaginal swab was collected for direct microscopic examination by wet mounts and Gram stains and another vaginal swab was used for aerobic cultures. One endocervical sample was collected in an Eppendorf tube containing 400 μL TE buffer and stored at -20ºC until molecular tests were conducted. The presence of syphilis was detected from the blood serum using a *Treponema pallidum* hemagglutination assay. A hysterosalpingogram was performed on all of the patients, and the information was combined with the microbiological results to analyze the risk factors.

Detection of genital tract infections

Bacterial vaginosis (BV)

A BV diagnosis was based on Amsel criteria when three of the following four characteristics were detected: vaginal pH of > 4.5, thin homogeneous discharge, clue cells, and/or "fishy" amine odor after the addition of 10% potassium hydroxide [1].

Gram staining was used under oil immersion (×l,000) to quantify the microbial morphotype of

Lactobacillus, *Gardnerella*, and other bacterial morphotypes such as Gram-negative bacilli, curved rods, and Gram-positive *cocci*. A smear was defined as "normal microbiota" when there was a microbial *Lactobacillus* morphotype with no other microbial morphotypes or with few *Gardnerella* morphotypes. A smear was defined as "mixed microbiota" when *Lactobacillus* morphotypes were present only in low numbers (1 to 2+), while a *Gardnerella* morphotype was present together with other Gram-negative and Gram-positive bacteria, such as curved rods, Gram-negative rods, fusiforms, and Gram-positive *cocci* [14].

Aerobic vaginitis

Aerobic vaginitis diagnosis was estimated by the clinical criteria with a change in the nature of the vagina and vaginal discharge and was combined with the positive culture test from the vaginal samples to detect aerobic bacterial pathogens [10,15]. The vaginal swab was rolled out onto blood agar, Drigalski lactose agar and chocolate agar plates (Liofilchem s.r.l., Teramo, Italy) to culture the aerobic bacteria and yeasts. The agar plates were examined after 24 hours and 48 hours. Additional bile esculin agar was used to differentiate the isolated *cocci*, and a commercial API 20E kit (bioMérieux SA, Lyon, France) was used for the bacilli isolates. Except for *Streptococcus agalactiae,* other bacterial pathogens associated with aerobic vaginitis such as *Enterococcus* spp., *Escherichia coli*, and *Staphylococcus aureus* were assessed in the culture diagnosis [15].

Candidiasis

The typical vaginal discharge was described as a cottage cheese-like discharge. The microscopic examination of the vaginal discharge with saline and 10% KOH allowed yeast identification as the presence of yeast blastospores or pseudohyphae. In addition, the presence of fungal colonies on the agar plates of the vaginal cultures were recorded [16].

Trichomoniasis

Microscopic observation of the motile protozoa from the wet mount of the vaginal samples was performed to diagnose trichomoniasis [17].

Chlamydia trachomatis

A phenol-chloroform DNA extraction method and a TaqMan probe-based real-time polymerase chain reaction (PCR) assay to detect *Chlamydia trachomatis* were performed using a commercial kit following the manufacturer's instructions (Viet A Corporation, Ho Chi Minh City, Vietnam).

Hysterosalpingogram (HSG)

A hysterosalpingogram (HSG) was performed by experienced practitioners in all of the cases to examine the uterine cavity and Fallopian tubes. First, a control image was obtained before the instillation of contrast (Ultravist 300 iopromide, Bayer, Reading, UK), approximately 10-20 ml each time, and a minimum of 3 more images were taken to examine the uterine cavity, tubal patency, and presence of contrast medium in the pelvic cavity.

Statistical methods

The analyses were performed using the Statistical Product and Service Solutions (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA). The continuous variables between the groups were compared using the independent sample t-test for normally distributed data or the Mann-Whitney U-test for skewed data. To compare the categorical variables, we used the chi-squared (χ^2) or Fisher's exact tests when appropriate. The results were expressed as the odds ratio (OR) with a 95% confidence interval (CI) or two-sided p-value. A p-value of < 0.05 was considered statistically significant.

Results

The age of the study population ranged from 20 to 45 with a mean age 31.0 ± 5.24 years and the duration of infertility ranged between 1 and 22 years with a mean duration of 3.6 ± 3.05 years. Of the 597 infertile women, 390 (65.3%) had primary infertility while 207 (34.7%) had secondary infertility.

The results of the microbiological analysis of the vaginal/cervical swabs of the women are shown in Table 1. In total, 307 vaginal/cervical swabs showed normal microbiota (51.4%), 31 (5.2%) showed mixed microbiota, and 259 (43.4%) showed infection. Single infection was more common than co-infection (39.5% *vs* 3.9%). Bacterial vaginosis was the most common diagnosis at 19.6% among the infertile patients, followed by candidiasis (17.4%). Only two of the 597 infertile women (0.3%) had trichomoniasis while *Chlamydia trachomatis* was present in 3.7% of the infertile women. As analyzed in the subgroups, the primary infertility group had a higher rate of *Vaginal bacteriosis* (31.7% *vs* 13.5%) and *Candidiasis* (27.8% *vs* 12.4%) than the secondary infertility group.

Table 1. Characteristics of genital tract infection with diagnosed pathogens (n = 597).

Diagnosis		Frequency			Incidence of pathogens (%)			p
		Infertility type		Infertile women	Infertility type		Infertile women	
		Primary	Secondary		Primary	Secondary		
Infection detected	n	174	85	259	N/A			0,188
	%	67.2	32.8	43.4				
Bacterial vaginosis	n	82	35	117	31.7	13.5	45.2	0.228
	%	70.1	29.9	19.6				
Candidiasis	n	72	32	104	27.8	12.4	40.2	0.357
	%	69.2	30.8	17.4				
Trichomoniasis	n	1	1	2	0.4	0.4	0.8	0.648
	%	50.0	50.0	0.3				
Chlamydial disease	n	18	4	22	6.9	1,6	8.5	0.098
	%	81.8	18.2	3.7				
Vaginitis	n	20	17	37	7.7	6.6	14.3	0.137
	%	54.1	45.9	6.2				
Single infection	n	N/A			155	81	236	N/A
	%				59.8	31.3	91.1	
Co-infection	n	N/A			19	4	23	
	%				7.3	1.6	8.9	
Mixed microbiota	n	23	8	31	N/A			
	%	74.2	25.8	5.2				
Normal microbiota	n	193	114	307	N/A			
	%	62.9	37.1	51.4				

N/A: Not Appropriate for analysis.

Table 2. Relation of general characteristics and genital tract infection by infertile types.

Characteristics	Primary infertility				p	Secondary infertility				p
	Infection (+)		Infection (-)			Infection (+)		Infection (-)		
	n	%	n	%		n	%	n	%	
Age										
< 35	148	444.0	188	556.0	0.574	45	40.5	66	59.5	0.870
≥ 35	26	48.1	28	51.9		40	41.7	56	58.3	
Infertility duration										
< 3 years	113	51.8	105	48.2	0.001	33	39.8	50	60.2	0.755
≥ 3 years	61	35.5	111	64.5		52	41.9	72	58.1	
History of GTI										
Yes	22	55.0	18	45.0	0.163	21	55.3	17	44.7	0.049
No	152	43.4	198	56.6		64	37.9	105	62.1	
Regular menstruation										
Yes	13	40.6	19	59.4	0.636	6	23.1	20	76.9	0.046
No	161	45.0	197	55.0		79	43.6	102	56.4	

GTI: genital tract infection.

Table 3. Hysterosalpingography results in infertile population.

Hysterosalpingography results	Total (n = 597)	Primary infertility (n = 390)	Secondary infertility (n = 207)	p
Normal patent tubes	522 (87.4)	358 (91.8)	164 (79.2)	0.000
Abnormal tubes	75 (12.6)	32 (8.2)	43 (20.8)	
Occlusion 1 side	32 (5.4)	15 (3.8)	17 (8.2)	
Occlusion 2 sides	33 (5.5)	12 (3.1)	21 (10.1)	
Hydrosalpinx 1 side	6 (1.0)	3 (0.8)	3 (1.5)	
Hydrosalpinx 2 sides	4 (0.7)	2 (0.5)	2 (1.0)	

Table 2 shows the relationship between genital tract infections and some clinical and demographic factors as classified between the primary and secondary infertility cases. In the primary infertility group, GTIs appeared to be associated with the duration of infertility (51.8% in < 3 years *vs* 35.5% in ≥ 3 years of infertility, p = 0.001), while in the secondary infertility group, GTIs appeared to be associated with a history of GTI (55.3% in the women with a history of GTI *vs* 37.9% in those without a history of GTI, p = 0.049) and menstrual disorders (23.1% in the women with regular menstruation *vs* 43.6% in those with irregular menstruation, p = 0.046).

Overall, 87.4% of the women had normal HSG results. Among the 12.6% with abnormal HSG findings, 5.5% had 2-sided tubal occlusions, 5.4% had 1-sided tubal occlusions, 1.0% had 1-sided hydrosalpinx, and 0.7% had 2-sided hydrosalpinx as shown in Table 3. There was a significant association between the hysterosalpingography results and the type of infertility (p < 0.001); 20.8% of the women with secondary infertility had abnormal tubes *vs* 8.2% of the women with primary infertility. In the bivariate analysis (Table 4), there were statistically significant associations between bacterial vaginosis and vaginitis and tubal diseases. In the infertility type subgroups, the women with secondary infertility with vaginitis had a higher rate of tubal diseases (47.1% *vs* 18.4%) than primary infertility, and BV appeared at a higher rate in the normal tubal group than (9.7% *vs* 2.4%) in the tubal disease group (p = 0.039).

Discussion

Our data showed that 43.4% of the infertile Vietnamese women in this study had some type of gynecologic infection. BV and candidiasis were the most common diseases followed by aerobic vaginitis, chlamydial disease, and trichomoniasis. Hysterosalpingography revealed abnormal findings in 12.6% of the women. No good correlations between HSG and infections were revealed in this study, except for aerobic vaginitis, which might increase the risk of tubal diseases.

The lower genital tract infections remain challenging in Vietnam because of their high prevalence and their role in infertility [9,10]. Previous studies reported similar infection rates, approximately 44% [18] or 45% [19]. However, in some studies, a somewhat lower prevalence was reported at 27.6% [20]. It is generally concluded that the rate of genital tract infections in infertile women appears to be higher than that of reproductive-age women in the general population [21].

Table 4. Bivariate association between specific infected pathogens and tubal disorders by infertility types.

Microbial infection	Total			Primary infertility			Secondary infertility		
	Abnormal HSG	Normal HSG	p	Abnormal HSG	Normal HSG	p	Abnormal HSG	Normal HSG	p
Genital tract infection									
Negative	45 (13.3)	293 (86.7)	0.527	23 (10.7)	193 (89.3)	0.063	22 (18.0)	100 (82.0)	0.296
Positive	30 (11.6)	229 (88.4)		9 (5.2)	165 (94.8)		21 (24.7)	64 (75.3)	
Bacterial vaginosis									
Non infected	68 (14.2)	412 (85.8)	0.019	30 (9.7)	278 (90.3)	0.039	38 (22.1)	134 (77.9)	0.366
Infected	7 (6.0)	110 (94.0)		2 (2.4)	80 (97.6)		5 (14.3)	30 (85.7)	
Candidiasis									
Non infected	60 (12.2)	433 (87.8)	0.517	25 (7.9)	293 (92.1)	0.634	35 (20.0)	140 (80.0)	0.487
Infected	15 (14.4)	89 (85.6)		7 (9.7)	65 (90.3)		8 (25.0)	24 (75.0)	
Vaginitis									
Non infected	66 (11.8)	494 (88.2)	0.037	31 (8.4)	339 (91.6)	0.592	35 (18.4)	155 (81.6)	0.010
Infected	9 (24.3)	28 (75.7)		1 (5.0)	19 (95.0)		8 (47.1)	9 (52.9)	
Trichomoniasis									
Non infected	75 (12.6)	520 (87.4)	N/A	32 (8.2)	357 (91.8)	N/A	43 (20.9)	163 (79.1)	N/A
Infected	0 (0.0)	2 (100.0)		0 (0.0)	1 (100.0)		0 (0.0)	1 (100.0)	
Chlamydial disease									
Non infected	75 (13.0)	500 (87.0)	N/A	32 (8.6)	340 (91.4)	N/A	43 (21.2)	160 (78.8)	N/A
Infected	0 (0.0)	22 (100.0)		0 (0.0)	18 (100.0)		0 (0.0)	4 (100.0)	

HSG: Hysterosalpingography; N/A: Not Appropriate for analysis.

Our study included sexually transmitted diseases such as chlamydial disease and trichomoniasis, but also non-STD vaginitis diseases such as BV, candidiasis, and aerobic vaginitis. The second group of diseases was much more frequent. *Chlamydia trachomatis* infection accounted for 3.7% of the patients in our infertility cohort. This obligate intracellular microorganism is the most common cause of pelvic inflammatory disease through damaging the columnar epithelium of the uterus and Fallopian tubes. The rate of chlamydial infection in this study was somewhat lower than in other reports [21,22]. The prevalence of another STD, trichomoniasis, was also very low in our study, which can be partly explained by the reported low prevalence in the general population of women in Vietnam [11,23]. Its role in female infertility has been understudied, but some investigators have suggested the possible association [2].

Bacterial vaginosis was the most common disorder in our study and was present in nearly one-fifth of the women. The disturbed vaginal communities in BV are considered a source of anaerobic bacteria-related pelvic inflammation that may be followed by chlamydia or gonorrhea infection. However, the association between bacterial vaginosis and infertility remains debatable [2,4,22]. Aerobic vaginitis is largely under-researched and its association with infertility is unclear. However, this condition is an important cause of adverse pregnancy outcomes [24]. There was no significant evidence relating *Candida* spp. with infertility; however, tubo-ovarian abscesses caused by this yeast infection have been described [25]. In our study, bacterial vaginosis and vaginitis were associated with the risk of abnormal HSG.

Our results showed that the rates of infections among the primary and secondary infertile women were 44.6% (174/390) and 41.1% (85/207), respectively. Tubal disease in infertile women is a common consequence of genital tract infections [6,7]. We used the non-invasive hysterosalpingogram test to evaluate the abnormalities of the uterus and Fallopian tubes in the infertile women in this study.

As an important result of our research, the population of infertile women displayed intact Fallopian tubes in the majority (87.4%) of cases, similar to the results of another reported study with Fallopian tube occlusion seen in only 11.2% [26]. Moreover, we found no substantial association between GTIs and tubal diseases. However, there was statistically significant difference between the subgroups of infertile women in cases of aerobic vaginitis, with greater risks in cases of secondary infertility (20.8% *vs* 8.2%). The development of pronounced tubal disease needs time and damaging infections can affect women with a history of abnormal HSG. On the contrary, the current infections may have lasted for a short time and might therefore not yet have been ascending and damaging the tubal tissue. Since aerobic vaginitis may frequently accompany STDs, it can be a consequence of previous or dormant STDs in these women, increasing the lifetime risk of tubal diseases.

Tubal occlusion is a common result of previous pelvic infections. It is indicated that infections are the most important cause of infertility [27]. Pelvic infections commonly lead to adhesions at the ampullary region of the Fallopian tubes, blocking the tubes. Hydrosalpinx is the consequence of an accumulation of inflammatory fluid in blocked Fallopian tubes [28]. In our study, tubal occlusion and hydrosalpinx were seen in 10.9% and 1.7% of the patients, respectively. Previous studies reported that the rate of abnormal HSG in tubal occlusion and hydrosalpinx was highly variable depending on the study population [26,29]; thus, further research is necessary.

PID is also associated with genital tract infections such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma genitalium* infections. However, delayed treatment of approximately 3 days in symptomatic women with PID was more likely to increase infertility than early treatment [30]. As a result, screening and early treatment for infections are the best ways to prevent infertility [31].

A limitation of our study includes focusing on some specific and common pathogens that can be examined using the available tests. We did not use the PCR test to detect *Trichomonas vaginalis* infection because of limited resources; instead, the diagnosis was made by wet mount with acceptable sensitivity and specificity [32].

The strength of our study lies in its prospective, cross-sectional design and consecutive sampling method. In addition, this epidemiological study described genital tract infections, focusing on infertile women in Vietnam. To date, local epidemiological studies of GTI remain limited in this region. Therefore, this study provides valuable information on the prevalence and distribution of genital tract infections as well as tubal diseases in reproductive-aged women presenting with infertility in Vietnam. The early diagnosis and appropriate management are both important to prevent the sequelae of pelvic inflammatory disease [33] and reduce the prevalence of female infertility.

Conclusion

Many infertile Vietnamese women have genital tract infections that can significantly influence their reproductive function and performance. These infections should be routinely screened and treated. In addition, sexual education should concentrate on preventive measures. These low-cost activities can provide significant benefits for avoiding both infections and their consequences, such as female infertility, which is especially important in developing countries.

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