Molecular Detection of Neisseria Gonorrhoeae and Chlamydia Trachomatis in an Infertile Woman

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Abstract
Background: The inability to conceive after a year of continuous, unprotected sexual activity is considered infertility, a reproductive condition that affects both men and women.
Aim: In the current investigation, bacterial vaginosis was isolated and identified using the real-time PCR technique to determine the influence of C. Trachomatis and N. Gonorrhoea infection as a risk factors for female infertility.
Methods: The research comprised 100 female infertility patients hospitalized in the hospital between March and June of 2022. One hundred samples of cervical swabs were collected. The sample was immediately kept at -20c until it was used at a later time to extract DNA.
Results: After the DNA was extracted from one hundred samples of infertile women to detect and quantify Neisseria gonorrhoeae using the Real-Time PCR technique using specific target sequences primers for the (16S rRNA gene 132 bp), the result shows that only 30 samples gave a positive result for N. gonorrhea. Also, to detect and quantify Chlamydia trachomatis using Real Time PCR technique using specific target sequence primers for the (16S rRNA gene 146 bp), the result shows that only 22 samples give a positive result for C. trachomatis.
Conclusion: Comparing RT-PCR to other approaches, it had a greater percentage of success in identifying N. gonorrhoeae and C. trachomatis in cervical samples.
Keywords: Infertility, Sexually transmitted diseases, Neisseria gonorrhoeae, and Chlamydia trachomatis

1. Introduction

The inability to become pregnant after a year of unprotected sexual activity is known as infertility, a condition of the reproductive system that affects both males and females(1). Infertility affects 15% of couples globally, with 40% of all instances involving female infertility(2). Female infertility has a complex cause. This includes endocrine abnormalities, ovulatory dysfunction, tubal and peritoneal illnesses, aging, which is linked to a loss in fertility, an increased chance of genetic mistakes in the developing embryo, and aging itself. Infections of the uterus are not frequent. The accumulation of behavioral risk factors, such as drinking, smoking or eating poorly (3). A significant proportion of infertility cases are still currently unsolved(4). Most women who struggle with infertility have inflammatory abnormalities in their oviducts or the surrounding peritoneum. (5). These conditions are usually caused by genital infections such as Chlamydia trachomatis and Neisseria gonorrhoeae(6). In healthy women, these bacteria may be commensal organisms. Still, they can also play a role in many pathologic diseases, including urogenital tract infections, unfavorable pregnancy outcomes, and unexplained infertility(7).
Most "unrelieved" disorders in the US are sexually transmitted diseases (STDs), widespread worldwide and particularly prevalent in the US, where 12 million new cases are reported annually. Surprisingly, STDs are the second reason for health loss among childbearing women and are a burden on public health in underdeveloped nations(8). A variety of pathogens and non-pathogen microorganisms may thrive in the female reproductive system. One of the most common causes of STDs in men and women, chlamydia infection, is closely linked to tubal factor infertility and a cause of pelvic inflammatory disease-related morbidity (i.e., infertility and ectopic pregnancy)(9). N. gonorrhoeae infection, like C. trachomatis, is also known to cause pelvic inflammatory disease, but both infections may be asymptomatic in some women, and many patients go undiagnosed and untreated(10). Both C. trachomatis and Neisseria gonorrhoeae are often detected in the genitourinary tract of healthy women and are considered opportunistic infections in humans. Both species, however, have been linked to higher chances of a few pathological diseases, such as bacterial vaginosis. (9). The problem of infertility has become a global problem that has spread in all countries, including Iraq. Because of the lack of studies on this problem in Iraq, we conducted this study, which dealt with the study of two of the most important causes of sexually transmitted diseases, which are considered one of the most important causes of infertility worldwide.

2. Materials and Methods

This cross-sectional comparative study has been done on 100 patients recruited from AL-Hilla for

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people attending infertility clinics between 18 – 45 years, from the end of March to Jun 2022. One hundred samples of cervical swabs were collected. The sample was immediately kept at -20c until it was used at a later time to extract DNA. DNA has been extracted from samples via "G-spin™/ Total DNA Extraction Mini Kit, iNtRON, Korea" the products purity and concentration also checked using a Nano-drop spectrophotometer, then have been stored at −20 ℃ until being used. The ethics of the Al-Hilla Teaching Hospital were followed in this research, and all subjects verbally consented after being given full disclosure.

Polymerase Chain Reaction (PCR)

The Real-Time PCR primer for direct detection and quantification of *Neisseria gonorrhoeae and Chlamydia trachomatis* were designed in this study using the NCBI Genbank sequence database, and these primers were synthesized by (Scientific Researcher Co. Ltd, Iraq) as the following table. Conditions of Real-Time PCR have adjusted as Pre-denaturation at 95 °C for 5 min, denaturation at 95°C for 20 sec, annealing at 60 °C for 1 min, detection (Scan) at 60 °C for 1 min, and Melt cycle at 65-90°C.

Table (1): Primer Sequence with their product size and references.

Primers		Sequence (5'-3')	Product size	Genbank
16S rRNA Neisseria gonorrhoeae	F	TTCGATGCAACGCGAAGAAC	132bp	AM921674.1
	R	AACCCAACATCTCACGACAC		
16S rRNA Chlamydia trachomati	F	TCACGTGTAGCGGTGAAATG	146bp	NR_025888.1
	R	ATGCATCGTTTACGGCAAGG		

3. Results

In the current study, after the DNA was extracted from one hundred samples of infertile women to detect and quantification Neisseria gonorrhoeae by using the Real-Time PCR technique using specific target sequences primers for the (16S rRNA gene 132 bp), the result shows that only 30 samples give a positive result for N. gonorrhoeae as illustrated in figure (1). Also, to detect and quantify Chlamydia trachomatis using the Real-Time PCR technique using specific target sequence primers for the (16S rRNA gene 146 bp), the result shows that only 22 samples give a positive result for C. trachomatis as illustrated in figure (2). The frequency distribution of female patients with infertility according to age groups is shown in table 2. The present study shows non-significant differences between the frequency distribution of N. gonorrhoeae and C. trachomatis according to age groups. Whereas N. gonorrhoeae shows a high-frequency distribution in age groups 20-29 years 13 (43.3 %) compared with other age groups, less than twenty years, 30-39 years, and more than forty years, 3 (10.0%), 10 (33.3 % and 4 (13.4 %) respectively. Also, C. trachomatis shows a high-frequency distribution in age groups 20-29 years 12 (54.6 %) compared with other age groups, less than twenty years, 30-39 years, and more than forty years, 2 (9.0 %), 7 (31.8%) and 1 (4.6%) respectively. The frequency distribution of female patients with infertility according to the type of infertility is shown in table 3.7. The present study shows non-significant differences between the frequency distribution of N. gonorrhoeae and C. trachomatis according to the type of infertility. Whereas N. gonorrhoeae show 13 (13.0 %) primary types of infertility and less than secondary types of infertility 17 (17.0 %), and C. trachomatis show only 8 (8.0 %) primary types of infertility and also less than secondary types of infertility 14 (14.0 %). The frequency distribution of female patients with infertility according to the duration of infertility is shown in table (3). The present study shows non-significant differences between the frequency distribution of N. gonorrhoeae and C. trachomatis according to the duration of infertility. Whereas N. gonorrhoeae show 11 (36.7 %) less than 3 years duration of infertility, 7 (23.3 %) between 3-5 years duration of infertility, 6 (20.0 %)between 6-9 years duration of infertility, and 6 (20.0 %) more than ten years duration of infertility. C. trachomatis show 9 (40.9 %) less than 3 years duration of infertility, 6 (27.3%) between 3-5 years duration of infertility, 4 (18.2 %) between 6-9 years duration of infertility, and 3 (13.6 %) more than ten years duration of infertility.

4. Discussion

The results of the current study to detect Neisseria gonorrhoea and Chlamydia trachomatis by Molecular detection by PCR showed 30 (30.0 %) and 22 (22.0 %), respectively. However, no significant differences (p-value = 0.400) were found in the molecular detection of Neisseria gonorrhoea and Chlamydia trachomatis, as illustrated in the following table (1). These results are expected in a society that commits traditions such as legal marriage. Still, in Western societies, that person has more than one sexual partner, and the rates of STDs (Neisseria and chlamydia) are higher.

Table (1): Identification of Neisseria gonorrhoeae, C. trachomatis and M. hominis among patients.

Age (years)	Total *n* (%)	Real Time PCR technique		*p*-value
		Positive n (%)	Negative n (%)	
N. gonorrhoeae, n (%)	100	30 (30.0 %)	70 (70.0 %)	0.400 ¥ NS
C. trachomatis, n (%)	100	22 (22.0 %)	78 (78.0 %)	
X2	1.833			
n: number of cases; SD: standard deviation; ¥: Chi-square test; S: significant at $p < 0.05$				

Table (2): Frequency distribution of N. gonorrhoeae, C. trachomatis and M. hominis according to Age groups.				
Bacteria species	Age groups			
	< 20, n (%)	20-29, n (%)	30-39, n (%)	≥ 40, n (%)
N. gonorrhoeae, n (%)	3 (10.0%)	13 (43.3%)	10 (33.3 %)	4 (13.4 %)
C. trachomatis, n (%)	2 (9.0 %)	11 (50.0 %)	8 (36.4%)	1 (4.6%)
p-value	0.792 ¥ NS			
X^2	3.133			
n: number of cases; SD: standard deviation; ¥: Chi-square test; NS: non-significant at p > 0.05				

Table (3): Comparison of frequency distribution of N. gonorrhoea and C. trachomatis according to type of infertility.				
Bacteria species	Positive n (%)	Type of infertility		p-value
		Primary n (%)	Secondary n (%)	
N. gonorrhoeae, n (%)	30 (30.0 %)	13 (13.0 %)	17 (17.0 %)	0.854 ¥ NS
C. trachomatis, n (%)	22 (22.0 %)	8 (8.0 %)	14 (14.0 %)	
X2	0.315			
n: number of cases; SD: standard deviation; ¥: Chi-square test; S: significant at p < 0.05				

Table (4): Frequency distribution of N. gonorrhoeae and C. trachomatis according to duration of infertility.				
Bacteria species	Duration of infertility			
	< 3, n (%)	3-5, n (%)	6-9, n (%)	≥ 10, n (%)
N. gonorrhoeae, n (%)	11 (36.7 %)	7 (23.3 %)	6 (20.0 %)	6 (20.0 %)
C. trachomatis, n (%)	9 (40.9 %)	6 (27.3%)	4 (18.2 %)	3 (13.6 %)
p-value	0.937 ¥ NS			
X2	1.804			
n: number of cases; SD: standard deviation; ¥: Chi-square test; NS: non-significant at p > 0.05				

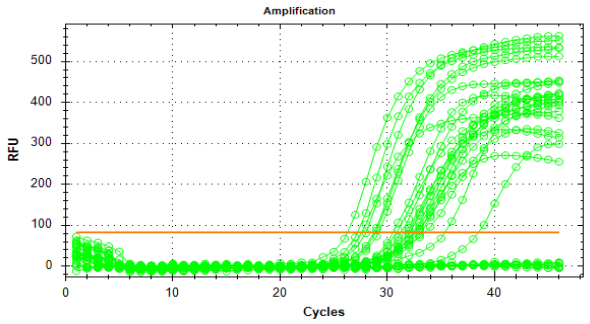

Figure (1): Real Time PCR amplification plots of 16S ribosomal RNA gene for detection Neisseria gonorrhea. samples

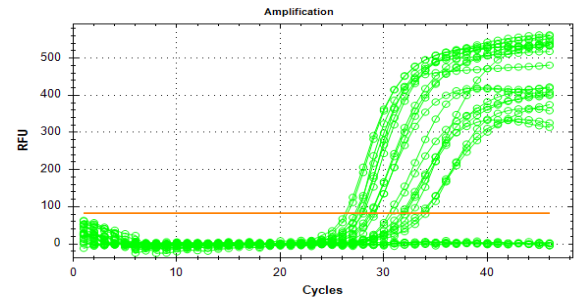

Figure (2): Real Time PCR amplification plots of 16S ribosomal RNA gene for detection Chlamydia sp. samples.

Following Chlamydia trachomatis, Neisseria gonorrhoeae is North America's second most frequent bacterial cause of sexually transmitted infections (STIs). (11). In European nations, the prevalence of gonorrhoeae has grown, rising from 8.2 cases per 100,000 people in 2008 to 23 cases per 100,000 people in 2017. Young people made up 36% of cases in the 23 European nations where gonorrhoea surveillance statistics were published by age, which is close to the percentage among 25 to 34-year-olds (37% of cases) (ECDC). Because N. gonorrhoeae may quickly resist several antibiotic classes, gonococcal infections are now a serious issue worldwide(12). Neisseria gonorrhoeae possesses proteins important for adhesion and pathogenicity. These proteins are Opa Opacity-Associated Proteins Adhesions between proteins in the outer membrane are numerous. These proteins have a role in intracolonial adherence of gonococci and extra colonial adherence to host cell receptors, such as heparin-related compounds and CD66 or carcinoembryonic antigen-related cell adhesion molecules. The outer membrane of the gonococcal contains part of the Opa molecule, while the remainder is exposed on the surface (13).

In young women, chlamydia infections are more common than in males; re-infection may occur asymptomatically and lead to the development of pelvic inflammatory disease, which can have consequences, including infertility. However, as many women have no symptoms, the infection may go unnoticed for a while (14). Evidence from infertile women suggests that asymptomatic salpingitis, in which a genital Chlamydia infection has been transferred, may also cause post-infectious tubal infertility in many women. Antigen-antibody interactions to Chlamydia heat-shock proteins have been proposed as a possible cause of morphological tissue damage and scarring, reducing fertility (15). These findings jibe with others showing a high prevalence of C. trachomatis infections in infertile women, suggesting that this bacterium is a major contributor to infertility worldwide (14). In several

publications, Infertile women have reported higher than average rates of Chlamydia infection (16).

Possible causes of the observed variation in infection rates include different detection technologies and socioeconomic environments. Previous bacterial infection was detected using the Real-Time PCR method in this research. However, in other studies, the difference in illness outcomes may have resulted from serological assays. Another important contributor to tubal infertility is subclinical infections, which are hard to identify clinically or by several laboratory tests that vary in their sensitivity and specificity. Thus, creating sensitive, precise, and speedy techniques for diagnosing these illnesses is strongly favored (17). Also, this result can be explained according to the type of society from which the samples were collected. These results are expected in a society that commits traditions such as legal marriage. Still, in Western societies, if a person has more than one sexual partner, the rates of STDs (Neisseria and chlamydia) infection are higher.

The present study shows that N. gonorrhoeae show a high-frequency distribution in age groups 20-29 years 13 (43.3 %) compared with other age groups, less than twenty years, 30-39 years, and more than forty years, 3 (10.0%), 10 (33.3 % and 4 (13.4 %) respectively. Also, C. trachomatis show a high-frequency distribution in age groups 20-29 years 12 (54.6 %) compared with other age groups, less than twenty years, 30-39 years and more than forty years, 2 (9.0 %), 7 (31.8%) and 1 (4.6%) respectively. The present results are consistent with the results of the CDC.,(18) Studies show that girls aged 20–24 are disproportionately affected by Neisseria gonorrhoeae (gonorrhea) (702.6 cases per 100,000 females). Gonorrhoeae infection rates declined 1.3% among those aged 15–19 and climbed 1.2% among those aged 20–24 in 2017–2018.

The most prevalent STI in the US is chlamydia trachomatis (chlamydia), and during the last several years, chlamydia cases have increased throughout the country. Increased usage of nucleic acid amplification tests (NAATs) between 2000 and 2011 may have contributed to the rise in cases observed. The increase in cases is likely the result of both an increase in incidence and changes in screening procedures. Young adults made up 61.8% of all cases in 2018; young women were disproportionately impacted, with instances increasing by 12.1% among females between the ages of 15 and 19 between 2014 and 2018. (18).

In contrast to several studies that found a higher prevalence of Chlamydia infections in women with primary infertility than in those with secondary infertility, there are no appreciable differences in the relationship between the incidence of Chlamydia infections and the types of infertility, which may be due to social factors and the types of screening tests used.

5. Conclusions

Our findings support that women in their 30s and 40s were the majority of patients with severe infertility. Comparing RT-PCR to other approaches, it had a greater percentage of success in identifying N. gonorrhoeae and C. trachomatis in cervical samples.

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