Percentage of Niesseria Gonorrhea among Symptomatic Women Attending Infertilty Clinic of Baghdad Teaching Hospital

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Abstract

Background: Niesseria Gonorrhea is a major cause of morbidity among sexually- active individual worldwide. The precise global burden of N. Gonorrhea is difficult to establish because of the lack of diagnostic capability and/or reporting system in many parts of the world.

Aim of the Study: To assess the percentage of Neisseria Gonorrhea in infertile women detected by conventional agar and polymerase chain reaction (PCR).

Study design: A cross sectional study

Setting: carried out in infertility clinic/Department of Obstetrics and Gynecology in Baghdad Teaching Hospital/Medical city for Ten months duration from the 1st of Feb 2017 to the 1ST of Dec 2017.

Patients and method: one hundred infertile women were included in the study. From each patient two endocervical sterile swabs were obtained.

Results: The mean age of the studied group was (27.8±4.2) years, more than one fourth (27%) of infertile women had previous history of sexually transmitted infection. Only 3 (3%) infertile women had positive culture test for Niesseria Gonorrhea while 17 (17%) infertile women had positive PCR test for Niesseria Gonorrhea. The validity results of PCR regarding diagnosis of Niesseria Gonorrhea in comparison to culture were sensitivity (100%), specificity (85.5%), +ve predictive value (82.4%), -ve predictive value (100%) and accuracy (86%)

Conclusion: The PCR is more accurate than culture for diagnoses of Niesseria Gonorrhea infection.

***Keywords:** Niesseria Gonorrhea, Female infertility, Endocervical swab*

Background

Neisseria Gonorrhea is a major cause of morbidity among sexually-active individuals worldwide. In the United States, it is the second most commonly reported communicable disease, with more than 350,000 cases reported annually, with probably an equal number of cases that remain unreported[1,2].Gonorrhea is a major cause of urethritis in men, cervicitis in women; the latter can result in pelvic inflammatory disease (PID), infertility, ectopic pregnancy, and chronic pelvic pain.

The growing threat of antimicrobial resistance in *N. Gonorrhea* highlights the importance of ensuring the availability of appropriate diagnostic modalities for surveillance [1]. The World Health Organization (WHO) has estimated the global incidence of several sexually transmitted infections (STIs) among individuals aged 15 to 49 years based on data from regions that have good case-based surveillance systems as well as data from population-based studies[3].

The highest incidence areas included Africa and the Western Pacific (including China and Australia) regions

[4]. The prevalence of gonorrhea infections reported in developing countries range from 2 –7 %[5]. In **Iran** in a study done by Afrasiabi S et al.[6], 2014 found that the prevalence of the N. gonorrhea was (2.38%), while Shokrollahi MR et al.,[7] study 2017, in **Saudi Arabia** mentioned that the prevalence of N. gonorrhea was (4.1%). The aim of this study is to assess the percentage of Neisseria Gonorrhea in symptomatic infertile women detected by conventional agar and PCR.

Patients and Method

Study design and setting

A cross sectional study carried out in infertility clinic /Obstetrics and Gynecology Department in Baghdad Teaching Hospital/Medical City for ten months duration from the 1st of Feb 2017 to the 1st of Dec 2017.(one day a week, four hours per day)

Sample size and sampling:

Endo cervical swab samples were obtained from 100 infertile women during the period of study. All the patients' details were taken, followed by pelvic exam. The inclusion and exclusion criteria of the study were marked. From each patient two endo cervical sterile swabs was obtained.

Principle of PCR Detection

Neisseria Gonorrhea DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Neisseria Gonorrhea* primers. The fluorescent dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermo cycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.M13-*opa*-Fw (TGT AAA ACG ACG GCC AGT GTT GAA ACA CCG CCC GG) and M13-*opa*-Rv (CAG GAA ACA GCT ATG ACC CGG TTT GAC CGG TTA AAA AAA GAT) primers (300 nM each) were used for amplification by PCR technique.

AmpliSens® Neisseria Gonorrhea-screen-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control-FL (IC)). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

AmpliSens® Neisseria Gonorrhea-screen-FRT PCR kit uses "hot-start," which greatly reduces the frequency of nonspecifically primed reactions. In variant FRT, "hot-start" is guaranteed by the separation of nucleotides and Taq-polymerase using a wax layer. Wax melts and reaction components mix only at 95 °C. In variant FRT-100 F, "hot-start" is guaranteed by the separation of nucleotides and Taq-polymerase using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

AmpliSens® Neisseria Gonorrhea-screen-FRT PCR kit is produced in 2 forms: AmpliSensÒ ® Neisseria Gonorrhea-screen-FRT PCR KIT variant FRTREF R-B51(RG)- CE, REF R-B51(iQ)-CE. AmpliSensÒ Neisseria Gonorrhea-screen- FRTPCR kit variant FRT-100 F REF R-B51-F(RG, iQ)-CE.

AmpliSens® Neisseria Gonorrhea-screen-FRT PCR kit variant FRT in

Data Analysis

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels: – The signal of the Neisseria Gonorrhea DNA amplification product is detected in the channel for the FAM fluorophore.

– The signal of the IC amplification product is detected in the channel for the JOE fluorophore. Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a Ct value of the DNA sample in the corresponding column of the results grid. Principle of interpretation is the following:

– Neisseria Gonorrhea DNA is **detected** in a sample if the Ct value is determined in the results grid in the channel for the FAM fluorophore. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of exponential growth of fluorescence.

– Neisseria Gonorrhea DNA is **not detected** if its Ct value is not determined (absent) in the results grid (the fluorescence curve does not cross the threshold line) in the channel for the FAM fluorophore, whereas the Ct

value determined in the results grid in the channel for the JOE fluorophore does not exceed the specified boundary value.

– The result is **invalid** if the Ct value is not determined (absent) in the channel for the FAM fluorophore, whereas the Ct value in the channel for the JOE fluorophore is not determined (absent) or exceeds specified boundary value. In such cases, PCR should be repeated for this sample.

The PCR was done in a private lab and by a Kit name (AmpliSensÒNeisseria Gonorrhea- screen-FRT PCR kit)(Manufactured by Ecoli s.r.o., Studenohorska 12 Russia)

Statistical Analysis

All patients' data entered using computerized statistical software; Statistical Package for Social Sciences (SPSS) version 21 was used. Descriptive statistics presented as (mean ± standard deviation) and frequencies as percentages. Kolmogorov Smirnov analysis verified the normality of the data set. One way ANOVA analysis was used to compare between more than two means. ROC curve was used to clarify validity tests. In all statistical analysis, level of significance (p value) set at ≤ 0.05.

Result

This study enrolled 100 infertile women with mean age of 27.8±4.2 years; 35% of them were 21-25 years age, 39% of them were 26-30 years age and 26% of them were 31-35 years age. Mean infertility duration of infertile women was 7.1±2.6 years; 9% of women had infertility duration of less than 5 years, 70% of women had infertility duration of 5-9 years and 21% of women had infertility duration of 10-14 years. About one third (27%) of infertile women had previous history of sexually transmitted infection (STI). Only 3 (3%) infertile women had positive culture test for Nisseria Gonorrhea while 17 (17%) infertile women had positive PCR test for Nisseria Gonorrhea. (***Table1***)

Table 1: Nisseria Gonorrhea culture and PCR findings of infertile women

Variable	No.	%
Culture results		
Positive	3	3.0
Negative	97	97.0
Total	100	100.0
PCR		
Positive	17	17.0
Negative	83	83.0
Total	100	100.0

No significant difference was observed between infertile women with positive PCR of Nisseria Gonorrhea and those with negative PCR regarding their age (p=0.1). No significant difference was observed between infertile women with positive PCR of Nisseria Gonorrhea and those with negative PCR regarding duration of infertility (p=0.2).(***Table2***) There was a significant association between infertile women with previous history of STI and positive PCR of Nisseria Gonorrhea (p=0.01).

Table 2: Distribution of infertile women age according to PCR results and history of STI

Variable	Positive PCR		Negative PCR		P
	No.	%	No.	%	
Age					0.1*NS
21-25 years	3	21.7	32	39.0	
26-30 years	11	56.5	28	33.8	
31-35 years	3	21.7	23	27.3	
Mean±SD (years)	28.7±4.2		27.4±4.1		0.2** NS
Variable	**Positive PCR**		**Negative PCR**		**P**
	No.	**%**	**No.**	**%**	
Duration of infertility					0.2*NS
<5 years	0	-	9	11.7	
5-9 years	15	78.3	55	67.5	
10-14 years	2	21.7	19	20.8	
Mean±SD	7.7±2.8		6.9±2.5		0.1** NS
Variable	**Positive PCR**		**Negative PCR**		**P**
	No.	**%**	**No.**	**%**	
Previous history of STI					
Positive	8	47.8	19	20.8	0.01*S
Negative	9	52.2	64	79.2	

**Chi-square test, **Independent sample t-test, NS=Not significant.*

No significant difference was observed between infertile women with positive culture of Nisseria Gonorrhea and those with negative culture regarding their age (p=0.3), however, mean age of infertile women with positive culture of Nisseria Gonorrhea was significantly higher than infertile women with negative culture. (***Table3***)

Table 3: Distribution of infertile women age according to culture results

Variable	Positive culture		Negative culture		P
	No.	%	No.	%	
Age					0.3*NS
21-25 years	0	-	35	35.2	
26-30 years	2	66.6	38	39.3	
31-35 years	1	33.3	27	26.5	
Mean±SD (years)	32.8±4.2		27.4±4		0.002** S

** Fishers exact test, **Independent sample t-test, S= Significant, NS=Not significant.*

No significant difference was observed between infertile women with positive culture of Neisseria Gonorrhea and those with negative culture regarding infertility duration (p=0.5), however, infertility duration of infertile women with positive culture of Nisseria Gonorrhea was significantly higher than infertile women with negative culture. Regarding to the +ve PCR in N. gonorrhea about 53% (9/17) were abnormal crevices and 47.0% (8/17) were within normal cervices.

Table 4: Relation between duration of infertility and culture results, and PCR distribution

Variable	Positive culture		Negative culture		P
	No.	%	No.	%	
Duration of infertility					0.5*NS
<5 years	0	-	9	9.6	
5-9 years	2	66.7	67	70.2	
10-14 years	1	33.3	21	20.2	
Mean±SD (years)	9.1±1.6		6.9±2.5		0.04** S

Variable	Abnormal cervices		Normal cervices		Total	
	No.	%	No.	%	No.	%
+ve PCR	9	53.0	8	47.0	17	100.0

** Fishers exact test, ** Independent sample t-test, S= Significant, NS=Not significant.*

No significant difference was observed between infertile women with positive culture of Nisseria Gonorrhea and those with negative culture regarding previous history of STI (p=0.1). The validity results of PCR regarding diagnosis of Nisseria Gonorrhea in comparison to culture were sensitivity (100%), specificity (85.5%), +ve predictive value (82.4%), -ve predictive value (100%) and accuracy (86%). (***Table 5***)

Table 5: Validity test results of PCR in comparison to culture in diagnosis of Nisseria Gonorrhea.

Validity test			Culture			
			Positive	**Negative**	**Total**	
			No. (%)	**No. (%)**	**No. (%)**	
PCR	Positive	No. (%)	3 (17.6)	14 (82.4)	17 (100.0)	
	Negative	No. (%)	0 (-)	83 (100.0)	83 (100.0)	
	Total	No. (%)	3 (6.0)	97 (31.7)	100 (100.0)	
Sensitivity		100%				
Specificity		85.5%				
+ve predictive value		82.4%				
-ve predictive value		100%				
Accuracy		86%				

Discussion

Infertility is a common public health concern worldwide; globally 9% of reproductive-aged women are infertile. The burden of infertility can reach up to 30% in reproductive-aged women. [8]

The mean age group of the patients in the current study was (27.8,±4.2 years) similar to the Shokrollahi MR study when it was (28.73±5.52).[8] Te Velde ER,[9] in the study carried on 2002 mentioned that the fertility of women reach peaks in the early and mid-20s years, after which it starts to decline, then this decline being accelerated after age 35. Which is in agreement with the current study in which it's revealed that about more than two third of the infertile patients was in the age below the 30 years old. [9] Likewise to the Afrasiabi S, 2014 in Iran when he assesses the frequency of the Endocervical infection of N. Gonorrhea among female carrier and changing trends of antimicrobial susceptibility patterns in Kashan, Iran. [6]

As mentioned by US preventive Task force, that most common age group infected with N. Gonorrhea was between 15-24 years old. [9] The current study mentioned that the majorities of the infertile women who infected with N. Gonorrhea and with +ve PCR results were in the age less than 30 years old,same that concluded by Kasanda G.[10] study in 2012 in Zambia to assess the prevalence and determination of N. Gonorrhea and C. trachomatis in PID patients when (58.6%) were in the age group of 20-29.

Regarding to the patients with history of sexual transmitted disease, it was found that 27 (27%) of the infertile women were previously infected with STD, less than that found by Kasanda G when the prevalence of STI were (32%).[10]

In Egyptian study done recently by Elkayal NM et al, in 2015 to detect of C.Trachomatis and N. Gonorrhea in women with infertility, this study was revealed that the PCR technique was the best and the gold standard method to detect the N. Gonorrhea in comparison with ELISA and culture, and the culture method is less sensitive than PCR for detection of the N. Gonorrhoeae. [11]This is in agreement with current study in which the validity results of PCR regarding diagnosis of Nisseria Gonorrhea in comparison to culture were sensitivity

(100%), specificity (85.5%). Likewise Gilson and Mindel,[12] study revealed that PCR have a sensitivity of at least 90% compared with 60% - 70% for culture. The sensitivity ofculture method in Gaydos CA, et al [13] study is (63.0%) and it is very close to Elkayal NM et al. [11] With same value, in a study carried by E.van Dyck, et al, [14] culture method shows (67.8%) sensitivity. Also, An Egyptian study found that the sensitivity and specificity of culture were 58.2% and 100%, respectively [14].

The present study revealed that there is high percentage of detection of the N. Gonorrhea infection by PCR (17%) than that by culture (3%) respectively, while in Rostami MN et al, [15] in 2017 the *N.Gonorrhoeae* infection was detected in 5 (1.2%) specimens by using culture and biochemical tests. Gaydos CA, et al, study in 2013, mentioned that in real-time PCR assay, only 17 (4.1%) were positive in detection of N. Gonorrhea infection.[16]This may be attributed tothe small sample size in our study (n=100), while in Rostami MN et al, study the sample size were (n=420) [14] and in Gaydos CA et al, in 2013 were [16] (1,722 female).

Conclusion

The PCR is more accurate than culture for diagnoses of Niesseria Gonorrhea infection. It is recommended that large sample size with enough period of time for future further study, and more than two samples supposed to be drawn from the patients.

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Conflict of Interest- Nil

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