RESEARCH ARTICLE

Diagnosis of *Neisseria gonorrhea* Using Molecular Method in Infertile Iraqi Male

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ABSTRACT

Background: Infertility is the inability of couples to have children after one year of marriage. There are many causes, including sexually transmitted diseases such as *Neisseria gonorrhea*, as these reasons can affect the characteristics of the semen.

Aim of the Study: The current study aimed to investigate the effect of STDs (*N. gonorrhoea*) infection on semen quality as a risk factor for male infertility.

Material and Methods: One hundred samples of sperm were obtained from infertile males, then analyzed using computer-assisted semen analysis (CASA), DNA extracted using a specific kit, and PCR performed.

Results: From the one hundred patients investigated for male infertility, it was found that (43%) primary and (57%) secondary infertility, the oligospermia (42%), asthenospermia (38%), oligo-asthenospermia (6%), normal (13%), Azoospermia (1%), while was detection DNA of *N. gonorrhea* 7% from isolates, respectively.

Conclusion: This investigation did not support the basic idea that *Neisseria gonorrhea* can cause male infertility. Moreover, no significant differences ($p < 0.05$) were supported between *N. gonorrh*ea and Seminal fluid characteristics.

Keywords: Asthenospermia, Male infertility, *Neisseria gonorrhea*, Oligospermia.

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INTRODUCTION

Infertility is defined as the inability to procreate after a year of unprotected sexual activity.[1] Primary and secondary infertility are the two types of infertility. Primary infertility is defined as a couple's inability to become pregnant after at least one year of sexual activity without the use of contraception. Couples who have been able to conceive at least once but have been unable to do so again have secondary infertility.[2]

According to certain studies, urogenital infections produced by infectious agents including bacteria, viruses, fungi, and protozoa are responsible for 15% of male infertility.[3]

Microorganisms can directly affect the male reproductive system by causing motile sperm agglutination, decreasing acrosome reaction capacity, and changing cell morphology, or indirectly by releasing reactive oxygen species produced by infection-induced inflammation, which has been linked to sperm DNA fragmentation and male infertility.[4]

Any guy can get asymptomatic genital tract infections, such as chlamydia infections, which can be detected in his seminal fluid. Another common source of STIs is gonorrhea, caused by the bacteria *Neisseria gonorrhoeae*.[5]

Infections of the urogenital tract are a common cause of infertility in men, and a variety of bacteria have been linked to infertility in men in various ways. Infections wreak havoc on reproduction in many ways. Damage to the spermatogenesis process, sperm dysfunction, and genital tract obstruction are examples of these processes. Chlamydia trachomatis, *N. gonorrheae*, and specific Mycoplasma pathogens, such as *Mycoplasma genitalia*, are the most common bacteria linked to male genital infections.[6]

The World Health Organization reports about 370 million STI infections per year. The pathogenic bacteria *N. gonorrhoea* are responsible for 87 million of them. The illness is one of the world's most deadly and rapidly spreading diseases.[7] Moreover, The second most common sexually transmitted disease is gonorrhea. Younger age, a new sex partner, a sex partner with concurrent partners, many sex partners, a history of gonorrhea infection, and having other sexually transmitted illnesses are the most common risk factor (Table 1).[7,8]

MATERIALS AND METHODS

Specimens Collection

One hundred samples of infertile men's semen were obtained by masturbation in a sterile plastic container from November 2020 to February 2021. Subjects were told to refrain from ejaculation for at least 72 hours before producing the semen

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sample. Before the standard semen process, the sample was liquefied for at least 20 minutes but no more than an hour; volume, pH, sperm concentration, sperm motility, and morphology were all measured as part of the investigation. In addition, there's direct microscopy. Following the analysis of the semen, a quantity of 2 mL of the liquid is packed in tubes and transferred to the laboratory, where it is frozen (-20°C) until it is utilized to extract the DNA later.

Microscopic Examination

For the microscopic inspection of semen samples, computer-aided semen analysis (CASA) technology was utilized to provide an automated and reliable assessment of individual sperm motility patterns in the semen.

DNA Extraction

Bacterial genomic DNA was extracted directly from the 100 semen samples using a specific extraction kit from Geneaid Company USA (No. GBB 101) and following the manufacturer's instructions.

Primers Design

The primers were designed for the detection of *Neisseria*. *Gonorrhea* out according to Promega company Korea is illustrated in Table 2.

Gel Electrophoresis

Analyze the amplification product by a 2% agarose gel with 1X TBE and dissolve it in a water bath at 100°C for 15 min, after which it was left to cool at 50°C; PCR products were visualized using the Gel Documentation System.

Statistical Analysis

The SPSS (27) program was used to detect the effect of different factors in study parameters. Least significant difference. Chi-square test was used to significantly compare between percentage p-value (0.05) probability in this study.

RESULTS

Semen Parameters

One hundred samples of semen were collected from men who have infertility, and they were from the age of 20 to 40 years. And through information from patients, the samples were divided into primary infertility and secondary infertility, primary infertility (43%), and secondary infertility (57%).

Regarding the relationship between infertility and Seminal fluid characteristics in this study, recorded a highly significant difference in the distribution of patients according to the level of significance ($p > 0.05$), as illustrated in Table 3.

Molecular Detection of *N. gonorrhoea* and *Chlamydia trachomatis*

After the DNA was extracted from 100 samples of semen from infertile men to detection the *Neisseria gonorrhea* by using the PCR technique by using specific target sequences primers for the (Opa gene 90 bp) as illustrated in Figure 1.

Present the current study results to detect *N. gonorrhea* by Molecular detection by PCR (7/100). However, no significant differences ($p < 0.05$) were found in the molecular detection of *N. gonorrhea*, as illustrated the following Table 4.

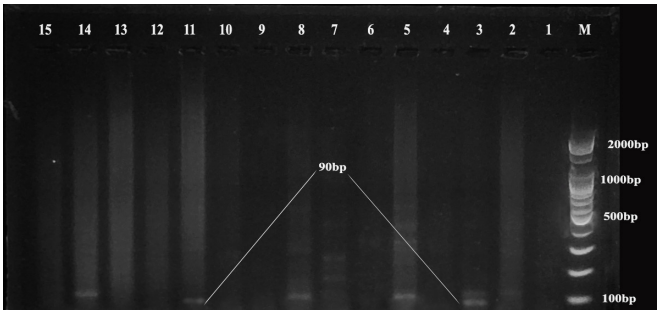

Figure 1: Agarose gel electrophoresis of PCR product obtained with *N. gonorrhoeae* strains using opa-specific primer, lanes 3 and 11 represent the identified *N. gonorrhoeae* with 90bp band, other lanes 1-15 (except 3 and 11) represent Negative results. Lane M represents 100 bp DNA ladder.

Table 1: Normal values of human seminal fluid (WHO).

Semen characteristics	*WHO 1992*	*WHO 1999*	*WHO 2010*
Volume (mL)	≥ 2	≥ 2	≥1.5
Sperm count (10^6/mL)	≥ 20	≥ 20	≥15
Total sperm count (10^6)	≥ 40	≥ 40	≥39
Total motility (%motile)	≥ 50	≥ 50	≥40
Progressive motility2	≥ 25% (grade a)	≥ 25% (grade a)	32% (a + b)
Vitality (% alive)	≥ 75	≥ 75	≥58
Morphology (% normal forms)	≥ (30)[4]	(14)[5]	4[6]
Leukocyte count (10^6/mL)	< 1.0	< 1.0	< 1.0

Table 2: The primers for the detection of *Neisseria. gonorrhea* out according to promega company

Neisseria gonorrheia genes				
Gene	*Primer sequences (5˚-3˚)*		*size (bp)*	*Reference*
Opa	F	TTGAAACACCGCCCGGAA	90	(Tabrizi *et al*., 2005)
	R	TTTCGGCTCCTTATTCGGTTTAA		

Table 3: PCR components used to identify *N. Gonorrhea* by the opa gene

Component	*Volume*
PCR Master mix	12.5 µL
DNA template 50ng	4 µL
Forward primer (10 pmol)	1.5 µL
Reveres primer (10 pmol)	1.5 µL
Nuclease free water	5.5 µL
Total volume	25 µL

PCR reaction

Neisseria .gonorrheia

Genes	*Step*	*T.M*	*Time*	*Cycle*
Opa (Detection)	Initial denaturation	95.0C	5 min	1
	Denaturation	95.0C	45 sec	40
	Annealing	54.7	45 sec	
	Extension	72.0C	45 sec	
	Final Extension	72.0C	10 min	1
	Hold	4.0 C	Forever	-

Table 3: Seminal fluid characteristics

Character	*Category*	*Number*	*%*	X^2	*p-value*
Semen volume	Normal (≥1.5)	98	98	184.32**	0
	Abnormal (<1.5)	2	2		
	Total	100	100		
Counting	Normal	51	51	101.44**	0
	Azoospermia	1	1		
	Oligospermia	42	42		
	Oligo-asthenospermia	6	6		
	Total	100	100		
Total motility	Normal	56	56	57.72**	0
	Oligo-asthenospermia	6	6		
	Asthenospermia	38	38		
	Total	100	100		
Pus cells/HPF	Normal (less than 1	0	0	200**	0
	Abnormal (more than 1)	100	100		

*No significant difference (p >0.05)
**Significant difference (p <0.05).

Table 4: Identification of *Neisseria gonorrhoeae* and *C. trachomatis* among patients male infertility.

Type of bacteria	*Total samples*	*Number*	*%*
Neisseria gonorhoea	100	7	7
X^2		0.579*	
p-value		0.447	

* No Significant difference (p >0.05)

Table 5: Association of type of infertility with a bacterial infection

Type of infertility	*Total No.*	*N. gonorrhea*	
		No.	*%*
Primary	43	4	9.3
Secondary	57	3	5.26
Total	100	7	7
X^2		0.614*	
p-value		0.433	

* No significant difference (p<0.05)

Regarding the relationship between *N. gonorrhea* the types of men's infertility, this study recorded no significant difference in the distribution of patients according to the types of infertility level of significance (p > 0.05), as illustrated in Table 5.

Table 6 shows the relationship between *Neisseria* with the total number of sperm, as a result, was no significant difference in the distribution of patients according to the types of infertility level of significance (p <0.05)

Table 6: Association of semen Counting with a bacterial infection

		N. gonorrhea	
Semen counting	*Total No.*	*No.*	*%*
Normal	51	2	3.92
Azoospermia	1	0	0
Oligospermia	42	4	9.52
Oligo-asthenospermia	6	1	16.66
Total	100	7	7
X^2		2.09*	
p-value		0.544	

* No Significant difference ($p > 0.05$)

Table 7: Association of sperm motility with a bacterial infection

		N. gonorrhea	
Total motility	*Total No.*	*No.*	*%*
Normal	56	4	7.14
Oligo-asthenospermia	6	1	16.66
asthenospermia	38	2	5.26
Total	100	7	7
X^2		1.039*	
p-value		0.595	

*No Significant difference ($p < 0.05$)

DISCUSSION

Primary infertility (43%) was less common than secondary infertility (57%). This study was close to a study conducted by,[9] contradicting a study[10] in this study recorded a significant difference in the distribution of patients according to the type of infertility at the level of significance ($p > 0.05$).

However, this result can be explained that in the case of primary infertility, genetic, chromosomal, and congenital factors have a more important influence than in the case of secondary infertility.[11] Also, many main causes of male infertility are urogenital infections, testicular failure, obstruction, testicular failure, low semen volume, agglutination of sperm, idiopathic infertility, varicocele erectile dysfunction, or ejaculation, abnormal viscosity, and endocrine disorder. High sperm density, congenital malformations, and environmental causes are significant causes of infertility. According to some research, idiopathic infertility accounts for 50% of male infertility cases. Moreover, the temperature is required for testicular function, which requires a temperature 2–4°C lower than body temperature. Fever above 38°C can affect spermatogenesis.[12]

Regarding the relationship between infertility and Seminal fluid characteristics (sperm count) in this study. The result was *Neisseria* (2/51 normal,4/42 oligospermia, and 1/6 oligo-asthenospermia, recorded a highly significant difference in the distribution of patients according to the level of significance ($p > 0.05$), as illustrated in Table 3.

Approximately 90% of male factor infertility is caused by either a low sperm count in the sperm or the generation of poor quality sperm reduced progressive motility, morphological defects.[13] Whereas the sperm parameters negatively correlate with more extended periods of infertility. These findings are consistent with the current investigation findings, which found that male patients with infertility for 1 to 5 years had the lowest proportion of sperm motility.[11]

Therefore, causes of male infertility can be classified into two categories: non-genetic factors, including diseases of the genitourinary system in particular (STDs), and genetic factors such as changes in chromosomes and congenital malformations. It affects the sperm directly or indirectly, which may limit the ability of these men to reproduce.[12] Seminal fluid infection increases with decreasing sperm density, motility, and morphology.

This finding can be interpreted in terms of the population from which the samples were taken. These findings are anticipated in a community that values traditions such as legal marriage; yet, in Western countries where people have several sexual partners, the prevalence of STDs such as *Neisseria* infection is higher, as illustrated the following of Table 4.

Note that there is a difference between primary and secondary infertility between *Neisseria*, as shown in the following Table 5. This figure shows the difference between the types of infertility with *Neisseria*. In contrast, that Primary infertility is more than secondary infertility in *Neisseria*.

These slight differences demonstrated in this study, although not significant, are still consistent with what has been observed in other studies that have shown an association between infertility types and *Neisseria*, as in the study.[14] The discrepancy in the proportions between primary and secondary infertility can depend on the study population, as it can vary from one population to another.

Table 6 shows the relationship between *N. gonorrhea* with the total number of sperm; as a result, was no significant difference in the distribution of patients according to the types of infertility level of significance ($p < 0.05$). The presence of a high rate of oligospermia and asthenozoospermia combined with the presence of pus cells can be explained by the bacterial strains present in the sperm, which can directly impact the quality of the sperm by reducing their motility. Such as *N. gonorrhoeae*, Mycoplasma, Ureaplasma, and Treponema. Pallidum is the most common sexually transmitted bacteria that affects semen.[6] Also, some types of negative bacteria, such as Bacillus coli and Klebsiella, and some positive bacteria, the most common of which are Staphylococcus aureus effect on semen parameters, as in many studies as the study by.[3,4,15,16]

However, compared to the total number of samples, we note no statistical significance. Moreover, the presence of *Neisseria* DNA in the sperm has been associated with poor sperm motility. In contrast, sperm motility and concentration.[17]

CONCLUSION

This study could not support our initial hypothesis that *Neisseria gonorrhea* can affect male infertility. As such, this study agrees with the bulk of previous studies that have concluded that semen quality is unaffected. Therefore, more work is required to dissect the precise sequence of events at

ejaculation if we are to fully understand the nature of any sperm damage for the fertility of men infected with *Neisseria*.

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