

The Prevalence of *Neisseria gonorrhoeae* among Iranian Women by Phenotyping and Genotyping Methods

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<http://dx.doi.org/10.22207/JPAM.10.4.25>

(Received: 30 August 2016; accepted: 28 September 2016)

Sexually transmitted disease (STD) consist of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, Syphilis and Trichomoniasis are a major public health concern. The prevalence of these organisms are increasing level because of homosexuality, migration and resistance to antibacterial agents and also usage of high technique for diagnosis of infection, according to WHO information 88 million people from 448 million which infected with STD, is *Neisseria gonorrhoeae*, whereas, Iran as a developing country have not complete prevalence information about *Neisseria gonorrhoeae*. In this study it has been tried to estimate the prevalence of *Neisseria* among Iranian women with phenotypin and genotyping methods. 300 specimens were enrolled in the present investigation which collected from women with genital problems. Dacron swab samples were cultured in chocolate agar in 37°C with 5-10% CO₂ for 24-48 hours. Gram staining, oxidase and carbohydrate utilization tests were used to identify the isolated species. Extraction of DNA of all isolates recognize as *Neisseria gonorrhoeae*. PCR for amplification of 390 bp fragment of CPPB plasmid for confirmation of isolates were performed. From 300 specimens, 7 isolates recognize as *Neisseria gonorrhoeae* with culture and PCR methods. It means *N. gonorrhoeae* was detected in 2.6% cases which enrolled in this study. The study revealed that although primary report found no prevalence of gonorrhoea in Iran but other study revealed different prevalence of bacteria in Iran, even in the recent studies, the results are very near to this result with consideration that gonococcal infection have different sequelae in young age or pregnant women so it is very important that with culture or PCR methods, screening of bacteria should be done.

Keywords: Phenotyping and Genotyping methods, STD, *Neisseria gonorrhoeae*.

The *Neisseriae* are gram-negative cocci that usually occur in pairs (diplococci). *Neisseria gonorrhoeae* (gonococci) is pathogenic for humans and typically are found associated with or inside polymorphonuclear cells. Gonococci attack mucous membranes of the genitourinary tract, eye, rectum, and throat, producing acute suppurative

that may lead to tissue invasion, this is followed by chronic inflammation and fibrosis. In women it causes genitourinary tract infection with sequelae of pelvic inflammatory disease, ectopic pregnancy^{1,2}.

The prevalence of organism has increasing level because of homosexuality, migration and resistance to antibacterial agents, according to WHO information, 88 million people from 448 million which infected with STD is *Neisseria gonorrhoeae*, from 1990s the prevalence

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grow up in most countries of the world , but differs from one country to another(3,4),information of iran is spars and spread , so in this study it has beeb tried to investigate the perevalenc of gonoroheae in 3 groups of women,group 1 with cervicitis,group 2 reffer for (D&C) and group 3 women with spontaneous abortion.in order to have more and correct information about the prevalence of *Neisseria gonorrhoeae* with using of phenotyping (culture)and genotyping(PCR) methods.

MATERIALS AND METHODS

During a 20 months study from September 2013 to March 2014, specimens were collected from 300 patients with genital problems who reffered to university of shahid beheshtie, Hospitals, Tehran,Iran.the specimens consist of 3 groups of women

Group 1: women with vaginal discharge ,abdominal pain ,bleeding (Cervicitis) .

Group 2: women who reffered to hospital for (D&C).

Group 3: pregnant women who reffered for spontaneous abortion . The mean age of group 1: was : 36year , group 2: 39year , group 3:27year.

Specimens were collected with 2 Dacron swab for each patients, one for stuart medium , the other for gram staining. All smear of gram stain were seen for extracellular or intracellular G-diplococcic. Specimens in stuart medium were

transport to chocolate agar and were placed in 37°C incubator, CO2 5% for 24-48 hours.Then oxidase test were done , for suspicious colonies utilaztion of sugars(glucose,lactose,maltose and sucrose) were used. (Table1,2).

For confirmation of isolates which recognize as *Neisseria gonorrhoeae*, extraction of 4.2 kb CPPB plasmid (5,6)by Nano plus plasmid Extraction kit BioNEER were done, PCR were performed with following format:

Primer Forward:5' GCT ACG CAT ACCCGC GTT GC3'

Primer Reverse:5' CGAAGACCTTCGAGCAGACA3'

PCR protocol :Primer forward=1µl,primer revers 1µl, DNA template 3

µl, Mastermix 12 µl, ,8µl H₂O → Totalvolume =25µl (94 °C 30sec,55°C 1min,74°C 30sec)94°C 5min

Lenght of product was 390bp by using 2% agaroze gel.

So the isolates which, with culture methods were *Neisseria gonorrhoeae* ,with molecular method were confirmed as *Neisseria gonorrhoeae* too.

RESULTS

300 specimens were enrolled in this study consist of 150 cervicitis,100 D&C,50 spontanous abortion ,53 specimens were Oxidase and Catalase positive.The result of utilization of sugares

Table 1. Result of Oxidaze test in3 groups

Oxidaze pos	Oxidaze Neg	G ·diplococci	Number	Specimen
23	29	52	150	Vaginal discharge
14	8	22	100	D&C
16	3	19	50	Spontaneous abortion

Table 2. Result of utilization of sugars in *Neisseria gonoroheae*

Sucrose		Maltose		Lactose	Glucose	Oxidase pos specimen	
20+	2-	8-	16+	2 -	20+	23+	Group 1=22
12+	2-	4-	10+	2 -	12+	14+	Group 2=14
14+	2-	2-	14+	2 -	14+	16+	Group 3=16

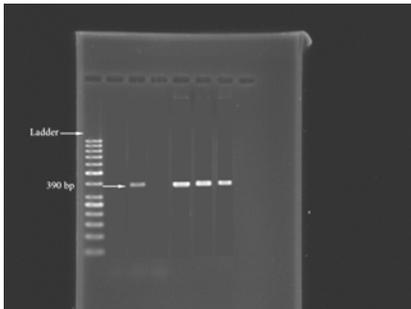
group 1=vaginal discharge

group 2=D&C

group3=spontaneous abortion

Table 3. Frequency of *Neisseria gonoroheae* among 3 groups

Frequency=%	Positive for <i>Neisseria gonoroheae</i>	Total number	Specimen
2%	N=3	150	group1=vaginal discharge
2%	N=2	100	group 2=D&C
4%	N=2	50	group3=spontaneous abortion

390bp band of *Neisseria gonoroheae*

according to table 2 revealed that 7 isolates were Glucose positive, Lactose, Maltose and Sucrose negative, which confirm that there were 7 isolates of *Neisseria gonoroheae* with phenotyping methods. Also with genotyping and molecular method and amplification of CPPB plasmid there were 7 isolates with 390bp band in PCR, which confirm of 7 isolates as *Neisseria gonoroheae*. So in our study with phenotyping and genotyping methods there were 7 isolates of *Neisseria gonoroheae*.

DISCUSSION AND CONCLUSION

This study sought to understand the prevalence of *Neisseria gonoroheae* among Iranian women who have genital problems by using of phenotyping (culture) and genotyping (PCR) methods. The result revealed that in 3 groups of women the prevalence of *Neisseria gonoroheae* was: group 1: 2%, group 2: 2%, and group 3: 4%. The mean of 3 groups was 2.6%. according to WHO information. The prevalence of *Neisseria gonoroheae* has an increasing level from 1990s and differs from one country to another, also these informations indicate that the prevalence of gonoroheae between 2005 to 2008 grow up to 21% (4). In USA, overall rate of gonoroheae was

111.6 per 100000 population between 1989-2008. (5,7). In Africa there are a review article which consist of studies about prevalence of STD infection among African men and women, they reports the prevalence of *Neisseria gonoroheae* in women 5%, and in high risk groups like sexworkers and women attending STIs clinics was between (10-31%). (6,8).

In Europe, a study has done with contribution of 12 European countries, an increasing rate in prevalence of *N. gonoroheae* observed from 1995 to 2002. In London, Wales and Northern Ireland gonoroheae diagnosed more than doubled between 1996 and 2001, in a study in Austria there was 414 cases of *N. gonoroheae* increase to 995 cases in 2002, with increasing rate both in men and women (7,9).

In neighborhood countries, like Turkey the Fahriye et al by using PCR found that the prevalence of gonoroheae was 3.7% among 370 endocervical samples (7,10), other study which has done in Palestine by using PCR and 213 endocervical specimens, revealed that the prevalence of gonoroheae was 1.4% (8,12), the other study in Bangladesh done by Rahman et al, found that prevalence of gonoroheae in women with vaginal discharge was 3.8% (9,13).

Studies which have done in recent years In Iran, cleared that in north west of Iran Zanjan province the prevalence of gonoroheae was 0.9% by using culture methods among pregnant and impregnant women. (10,14), in Babol north of Iran the prevalence was 0.2% by culture method (11,15), in Sabzevar the prevalence was 0.9% by using triplex PCR (12,16), in Kermanshah west of Iran, the overall incidence of gonoroheae was 2.4% among 255 married women by doing PCR. (13,17), the other study which has done in Kashan found the prevalence of gonoroheae 2.38% among 294 married women and using culture

method(14,18) these studies which has done in recent years have similar and near result, specially the result of Afrasiabi,(Kashan) and Akyaa (Kermanshah) confirm our study ,but in a study which performed by Batool Hossein Rashid et al ,in Tehran,they examined 209 infertile women as cases and 170 pregnant women as controls by using PCR for detection bacteria in urine ,they found no gonorrhoeae infection among the society which were selected(15,19),their result is opposite to other study which has done in Iran , different result could be because of the source of specimen which they selected, it is likely that vaginal and genital discharge are better source for searching bacteria in women than urine. with consideration that young women and female adolescents are more susceptible to STIs compared to the male due to their anatomy , so infection in women could have different sequelae like ectopic pregnancy or pelvic inflammatory disease (PID),it is better to culture of vaginal discharge become a routine test for women especially in young age for diagnosis and treatment of gonococcal infection .

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