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Pelvic Inflammatory Disease With Special Reference To *Mycoplasma Genitalium* in A Medical College and Hospital, Kolkata

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ABSTRACT

Mycoplasma genitalium , a pathogenic bacterium which lives on the ciliated epithelial cells of the urinary and genital tracts in humans . It was first identified in the early 1980s among men with non-gonococcal urethritis.The study was conducted on 50 women, divided into two groups: 25 cases with Clinically suspected Pelvic Inflammatory Disease (study group) and 25 healthy women (control group). Three cervical swabs were collected from each case and sent for bacteriological examination for mycoplasmas. Present study was conducted from August 2012 to December 2015 in a Medical College and Hospital, Kolkata for the detection of M. genitalium. 6 specimens were PCR positive for Mycoplasma genitalium .From the Control group none of the specimens were PCR positive for Mycoplasma genitalium. From the 6 women from whom M. genitalium was isolated their husband's urethral swabs were also collected and shown PCR positive for Mycoplasma genitalium. Among this six women four belongs to age group 20 to 30 years where as two from 30-40 years.

KEYWORDS : *Mycoplasma genitalium*, Cervicitis, Pelvic inflammatory disease (PID), PCR

Introduction

Mycoplasma genitalium , a pathogenic bacterium which lives on the ciliated epithelial cells of the urinary and genital tracts in humans . It was first identified in the early 1980s among men with non-gonococcal urethritis[1]. ***Mycoplasma genitalium*** is very difficult to culture, only with polymerase chain reaction (PCR) technology the research of M genitalium pathogenicity has been proven. Numerous studies have confirmed M genitalium drug resistant non-gonococcal urethritis, and some have also linked M genitalium with cervicitis independent of Chlamydia trachomatis and Neisseria gonorrhoeae. Cervicitis is also a risk factor for pelvic inflammatory disease (PID)—the common infection and inflammation of the upper genital tract of females, which can cause major reproductive morbidity including infertility.[2-5]. *M. genitalium* is associated with cervicitis and cervical inflammation . As *C. trachomatis* is a common cause of cervicitis and due to this reason may confound this series of studies, some have not considered patients testing positive for *C. trachomatis* or have adjusted for it in multivariate analyses. The vast majority of these have shown an independent, significant association between *M. genitalium* and cervicitis [5]. The pathogenic role of *M. genitalium* is less definitive in women than it is in men. *M. genitalium* can be found in the vagina, cervix, and endometrium and, like chlamydial and gonococcal infections, *M. genitalium* infections in women are commonly asymptomatic. *M. genitalium* can be detected in 10%–30% of women with clinical cervicitis, and most (6-12)studies have found that this organism is more common among women with cervicitis than those without this syndrome (13-15). *M. genitalium* is found in the cervix and/or endometrium of women with PID more often than in women without PID *(6-10)*, and endosalpingitis develops in nonhuman primates after inoculation with *M. genitalium*, suggesting that this organism can cause PID. *M. genitalium* has been detected in 2%–22% of PID cases (median: 10%) depending on the setting, but the frequency with which *M. genitalium*-infected women experience PID has been under studied. Although one study in Sweden reported a substantial increase in risk for postabortal PID among women with *M. genitalium*, the proportion of *M. genitalium*-positive women who subsequently experienced PID in two other studies was relatively low (<5%) (17,18), and evidence from serologic studies assessing the association of PID with antibody to *M. genitalium* is inconsistent. Overall, evidence suggests that *M. genitalium* can cause PID, but that this occurs less frequently than it does with *C. trachomatis* (16-18).

Materials and Methods

The study was conducted on 50 women, divided into two groups: 25 cases with Clinically suspected Pelvic Inflammatory Disease (study group) and 25 healthy women (control group). Three cervical swabs were collected from each case and sent for bacteriological examination for mycoplasmas. Present study was conducted from August 2012 to December 2015 in a Medical College and Hospital,

Kolkata. For the detection of M. genitalium, the endocervical specimenwas inserted in a buffer solution, using Cobas Amplicor specimen transport medium collection tubes(Roche Diagnostic Systems). The samples were stored at 4°C until transport to the Microbiology Departmental laboratories within 12 hours of collection. These specimen were kept at -20°C until sample collection from 30 specimen were completed. Culture for genital mycoplasmas. Specimens were inoculated onto A7agar(Becton Dickinson, Cockeysville, Md. 21030) and incubated at 37°C in 5% CO2 for 5days. For Urea plasma it is inoculated in 10% urea supplemented broth. Cultures were examined microscopically daily for 5 days for the appearance of typical mycoplasma colonies. Specimens were also inoculated in Urogenital Mycoplasma broth incorporated with yeast extract, Horse Serum, vitamin and minerallo growth supplements and then followed by subculture in to A7agar. Multiplex PCR assay for genital mycoplasma infection. Bacterial DNA from100 micolitre of specimen or transport media was isolated by lysis in 400 micro litre of lysis buffer, extracted with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1), and extracted again with chloroform-isoamyl alcohol. DNA was then precipitated in 100% isopropanol, washed in 70% ethanol, and suspended in 15 micro litre of RNase-DNase free sterile deionized water (Sigma, St. Louis, Mo.). PCR was performed with primers specific for the 140-kDa adhesion protein gene of M. genitalium.50microlitre reactions containing a 0.2 mM concentration of de-oxynucleoside triphosphate mixture,10mM Tris, 3mM MgCl2, 25pmol of each unlabeled forward primers, and 25pmol of biotin-labeledreverse primer (Table1) and 1.25U of Gold Taq (Applied Biosystems.). All reactions were performed in a Thermo cycycler under the:

FOLLOWING CONDITIONS

First cycle at 95°C for 10 minutes, after that at 95°C, 35 twostep cy-

cles for 15s and 60°C for 60s, after that 5min at 72°C for PCR product detection. Enzyme-linked oligosorbent assay (ELOSA) was used for the detection of the PCR products of *M. genitalium*.The analytical sensitivity was determined by amplification of twofold serial dilutions of DNA of the bacteria, either individually or all three organisms as a mixture. 3.13 to 100CFU dilutions were done. The

CFU equivalent of DNA in the last sample positive in the dilution series was the lower limit of detection (LOD).

Table:1

TABLE 1. Nucleotide sequences of primers and probes used

Analysis, organism, and primer or probe	Target or DNA sequence (5′–3′)	Length (bp)
Mycoplasma genitalium	MG16-45 F TACATG-CAGTCGATCGGAAGTAGC	282bp
Mycoplasma genitalium	MG16-447R AAACTCCGC-CATTGCCTGCCTGCTAG	282bp

Result

The study was conducted on 50 women, divided into two groups: 25 cases with Clinically suspected Pelvic Inflammatory Disease (study group) and 25 healthy women (control group).From the clinically suspected Pelvic Inflammatory Disease (study group) 6 specimens were PCR positive for *Mycoplasma genitalium* .From the Control group none of the specimens were PCR positive for *Mycoplasma genitalium*. From the 6 women from whom *M. genitalium* was isolated their husband's urethral swabs were also collected and shown PCR positive for *Mycoplasma genitalium*. Among this six women four belongs to age group 20 to 30 years where as two from 30-40 years.

Discussion

M. genitalium is isolated from the cervix and endometrium of women with PID more often than in women without PID *(6-10)*, and endosalpingitis develops in nonhuman primates after inoculation with *M. genitalium*, suggesting that this organism can cause PID. *M. genitalium* has been detected in 24% of the symptomatic patients and treated. It saved the complications like formation of scar tissue both outside and inside the fallopian tubes that can lead to tubal blockage;ectopic pregnancy (pregnancy outside the womb);infertility (inability to get pregnant);long-term pelvic/abdominal pain. From the healthy women no *M. genitalium* was isolated .It again proves the pathogenic role of *M. genitalium* [2-5].

Conclusion

All the six women's husbands urethral swabs were also collected and shown PCR positive for *Mycoplasma genitalium*. Which proves the sexually transmissible role of *Mycoplasma genitalium*. The relation of *M. genitalium* PID and infertility are quite true and indicate that this organism has capability to cause ascending infection but more studies are needed to understand the relationship between *M. genitalium* and urogenital disease in women. Among this six women four belongs to age group 20 to 30 years where as two from 30-40 years which indicates its prevalence in sexually active women and their partners. In conclusion, *M. genitalium* is an important cause of sexually transmitted infections in both men and women but more studies required to confirm it.

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