Infertility with Special Reference to Genital Mycoplasmas in a Medical College and Hospital Kolkata

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ABSTRACT

Introduction: In humans, infertility may describe a woman who is unable to conceive as well as being unable to carry a pregnancy to full term. Mycoplasma and Ureaplasma are the micro-organisms of sexually transmitted diseases. Several epidemiological reports have documented the presence of M. hominis, M. genitalium and U. Urealyticum in infertile women. Also in vitro studies have shown that sperm samples infected with these Mycoplasmas undergo detrimental changes in sperm count, sperm velocity and motility parameters.

Material and methods: The study was conducted on 100 women, divided into two groups: 60 cases with unexplained infertility (study group) and 40 cases with confirmed fertility (control group). Three cervical swabs were collected from each case and sent for bacteriological examination for mycoplasmas. Culture for genital mycoplasmas. Specimens were inoculated onto A7agar(Becton Dickinson). Bacterial DNA from100micolitre of specimen or transport media was isolated. Multiplex PCR was performed with primers specific for highly conserved regions in the ureasegene of Urea plasma spp, the 140-kDa adhesion protein gene of M. genitalium, and the 16Sr RNA gene of M. Hominis.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-labeled reverse primer (Table1) and 1.25U of GoldTaq (Applied Biosystems.). All reactions were performed in a Thermocycycler.

Results: Mycoplasmas were isolated from 14 cases (28%) in study group and two cases (4%) in the control group. Out of these, 6 cases and 1 case in the study and control group respectively were positive for U. urealyticum.

Conclusion: Many types of genital infectious diseases (such as cervicitis, pelvis inflammatory disease) are caused by M. hominis and U. urealyticum, for infertility, but their actual role in obstetrical pathologies (premature delivery, premature rupture of membranes, chorio-amniotitis) and neonatal infections has not been proven. Doubts about their role still exist whether these mycoplasmas are pathogens or mere co-factors associated with genital infections and more studies need to be done to confirm their role.

Keywords: Genital Mycoplasmas

INTRODUCTION

In humans, infertility may describe a woman who is unable to conceive as well as being unable to carry a pregnancy to full term. There are many biological and other causes of infertility, including some that medical intervention can treat.[1] Women reproductive system harbours various pathogen and non pathogen microorganisms. Mycoplasmataceae is a family of bacteria which cause urogenital infections and the complications caused by these bacteria may lead to infertility in women.[2] Mycoplasma and Ureaplasma are the microorganisms of sexually transmitted diseases. They are considered to be a threat to Public health.[3] Most of these infections are not diagnosable due to lack of symptoms, the antibacterial effect of sperm, the higher possibilities of contamination with other urethral organisms and the difficulty of culturing.[3,4] Numerous researchers have attempted to study the association between genital Mycoplasmal infections and infertility. Several epidemiological reports have documented the presence of M. hominis, M. genitalium and U. urealyticumin infertile women.[5,6] Also in vitro studies have shown that sperm samples infected with these Mycoplasmas undergo detrimental changes in sperm count, sperm velocity and motility parameters.[7] The pregnancy success rate of in vitro fertilization (IVF) might be reduced as a result of prior mycoplasma colonization of the female and male genital tract.[3]

MATERIALS AND METHODS

The study was conducted on 100 women, divided into two groups: 60 cases with unexplained infertility (study group) and 40 cases with confirmed fertility (control group). Three cervical swabs were collected from each case and sent for bacteriological examination for mycoplasmas. Inclusion criteria for the study group is unexplained infertility and exclusion criteria is confirmed fertility and its just reverse for the control group. Present study was conducted from August 2012 to August 2014 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°Cuntil transport to the Microbiology Dept.laboratories within 12 hours of collection. These specimen were kept at -20°C until sample collectionfrom 30 specimen were completed. Culture for genital mycoplasmas. Specimens were inoculated onto A7agar(Becton Dickinson, Cockeysville, Md. 21030) and

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Analysis, organism, and primer or probe	Target or DNA sequence (5'–3')	Length (bp)
Mycoplasma hominis	RNAH1 CAATGGTAATGCCGGATACGC	334bp
Mycoplasma hominis	RNAH2 GGTCCGTCAGTCTGCAAT	334bp
Mycoplasma genitalium	MG16-45 F TACATGCAGTCGATCGGAAGTAGC	282bp
Mycoplasma genitalium	MG16-447R AAACTCCGCCATTGCCTGCCTGCTAG	282bp
Ureaplasma urealyticum	U4 primerACGACGTCCTAAGCACT	429bp
Ureaplasma urealyticum	U5 primer CAATCTGCTCGTGGTATTAC	429bp

Table-1: Nucleotide sequences of primers and probes used

incubated at 37°C in 5% CO2 for 5days. For Urea plasma it is inoculated in 10% urea supplemented broth. Cultures were examinedmicroscopically daily for 5days for the appearance of typical mycoplasma colonies. A7agar incorporates a direct test for urease that allows the differentiation of ureaplasma from the other Mycoplasma tales. Specimens were also inoculated in Urogenital Mycoplasma brothincorporated with yeast extract, Horse Serum, vitamin and minerallo growth supplements and thenfollowed 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.). Multiplex PCR was performed with primers specific for highly conserved regions in the ureasegene of Ureaplasma spp, the 140-kDa adhesion protein gene of M. genitalium, and the 16Sr RNA geneof M. Hominis.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 two-step cycles 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 Urea plasma and M. genitalium. Further evaluation by digestion with NarI was done for M. hominis, which results in the digestion of M.hominis PCR product to fragments of 62 and 272bp Analytical sensitivity. 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).

RESULTS

Mycoplasmas were isolated from 14 cases (28%) in study group and two cases (4%) in the control group. Out of these, 6 cases and 1 case in the study and control group respectively were positive for U. urealyticum and the difference was statistically highly significant. Mycoplasma hominis was found to be positive in seven cases and one case in the study and control group respectively and was statistically insignificant ($P > 0.05$). The colonization of mycoplasmas was maximum in the age group 26-30 years and low socio economic group. Mycoplasma genitalium was found to be positive in one case and no case in the study and control group respectively.

DISCUSSION

Many researchers have attempted to study the role of genital Mycoplasma infections and infertility. Several epidemiological reports have documented the presence ofM. hominis,M. genitalium and U. urealyticumin infertile women.[5,6] Our study also go with these researchers findings.M. genitalium likely maintains persistent infection through intracellular survival in mucosal epithelial cells.[8,9] resulting in inflammation.[8,10] The observed correlations between M. genitalium reproductive tract infection and infertility may be explained by long-term inflammation elicited by M. genitaliuminfection.[10] From the uterine cervix of infertile women attempts were made to isolate mycoplasmas and normal pregnant and nonpregnant women to investigate the relationship of uro -genital mycoplasma infection to infertility. Ureaplasma urealyticum and M.hominis were isolated.[11] Female genital tract infections are one of the reasons a of infertility. Gnarpe and Friberg first suggested an etiologic role of Mycoplasma in infertility by demonstrating a high frequency of positive cultures recovered from the cervices of women with unexplained infertility compared with those of the fertile pregnant control subjects. DeLouvois et al. studied 120 patients with infertility of various etiologies and found a 52% incidence of cervical ureaplasma. They also found a 55% incidence in 92 pregnant patients. Matthews et al. and Nagata et al. 9 found similar results. Gump et al. studied 20 patients with infertility for longer than year and obtained cultures from the cervix and endometrium for Mycoplasma and Ureaplasma.[12-15]

CONCLUSION

Many types of genital infectious diseases (such as cervicitis, pelvis inflammatory disease) are caused by M. hominis and U. urealyticum, for infertility, but their actual role in obstetrical pathologies (premature delivery, premature rupture of membranes, chorio-amniotitis) and neonatal infections has not been proven. Doubts about their role still exist whether these mycoplasmas are pathogens or mere co-factors associated with genital infections. M. genitalium has been proven

pathogen of genital tract; new studies will be necessary so that one has a better understanding of the pathologies it can induce.Our study also shows their certain role in infertility and more study need to be done to confirm their role in infertility

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