Iranian Journal of Microbiology
Volume 6 Number 4 (August 2014) 219-224

ORIGINAL ARTICLE

Colonization of *Mycoplasma hominis* and *Ureaplasma urealyticum* in pregnant women and their transmission to offspring

Behnam Sobouti[1], Shahrzad Fallah [2], Mohammadreza Mobayen[3], Samileh Noorbakhsh[4], Yaser Ghavami[5*]

[1]Infectious Disease Research Center, Ali-Asghar Children Hospital, Iran University of Medical Sciences, Tehran, Iran. [2]Shahid Beheshti University of Medical Sciences,Tehran, Iran. [3]Tehran University of Medical Sciences, Tehran, Iran. [4]Infectious Disease Research Center, Rasoul Akram Hospital, Tehran, Iran. [5]Shahid Motahari Burn Research Center, Iran University of Medical Sciences, Tehran, Iran.

Received: August 2013, **Accepted:** June 2014

ABSTRACT

Background and Objectives: *Mycoplasma hominis* and *Ureaplasma urealyticum* are important opportunistic pathogens that cause urogenital infections and accelerated newborn delivery in pregnant women. Moreover genital mycoplasmas have been implicated in different neonatal diseases such as pneumonia, sepsis and meningitis. This study was conducted to find out the prevalence and transmission rate of these two organisms in pregnant women and their neonates.

Materials and Methods: Nasotracheal and pharyngeal specimens of 165 newborns hospitalized at Neonatal Intensive Care Unit (NICU) of Rasoul Akram Hospital (during 2010 – 2011) were assessed by PCR to detect *M. hominis* and *U. urealyticum*. Moreover, PCR of vaginal specimens from their mothers were obtained to determine the prevalence of these organisms in pregnant women and rate of transmission to their newborns. Data were analyzed using SPSS software.

Results: Totally, the results of PCR were positive in 33 newborns (20%). Vaginal colonization among the mothers was found to be 15% (25/165) for *U. urealyticum* and 15% (25/165) for *M. hominis*. The transmission rate to their infants was 72% and 60% for *U. urealyticum* and *M. hominis*, respectively.

Conclusion: These data indicate that vertical transmission of mycoplasma and ureaplasma are prevalent in newborns. Since these organisms cause serious infections in neonates, it would be better to perform screening tests in pregnant women before the delivery in order to prevent transmission to neonates and consequent infections and morbidities among them.

Keywords: *Mycoplasma hominis; Ureaplasma urealyticum*; Transmission Rate, Pregnant women

INTRODUCTION

Female's genital is a proper site for growth and proliferation of various organisms. Microorganisms can affect any part of genitourinary tract and cause infections. Moreover their complications such as ectopic pregnancy, fallopian tube obstruction, infertility, frequent abortions and cervical dysplasia are devastating (1, 2). Amongst numerous organisms which can be found in female's reproductive system, *M. hominis* and *U. urealyticum* are frequently isolated. In previous decades, diagnosis and research on these microorganisms were more prominent since they could cause serious infections both in mothers and newborns. Although they are still important pathogens, but currently they are not in the top list of

*Corresponding Author: Yaser Ghavami MD. Shahid Motahari Burn Research Center, Iran University of Medical Sciences, Tehran, Iran
Email: ghavamiy@gmail.com
Tel +98-21-88770031

Table 1. Target for detection of seeplex organism detection assay by multiplex PCR assays

Seeplex organisms detection assay	Target	Accession number	Amplicon size (bp)	Primer
Arabidopsis (internal control)	Cesa3		719	N/A
***Ureaplasma* urealyticum**	UreG-D	AF085729	348, 435, 502	**UMS125** GTATTTGCAATCTTTATATGTTTTCG **UMA226** CAGCTGATGTAAGTGCAGCATTAAATTC
***Mycoplasma* hominis**	Gap	AJ243692	214	**RNAH1 primer** CAATGGCTAATGCCGGATACGC **RNAH2 primer** GGTACCGTCAGTCTGCAAT

interest for researchers. Mycoplasma species are the smallest and simplest organisms which can live and proliferate freely in artificial media. *U. urealyticum* is one of the most important organisms which can be isolated from lower respiratory tract and nervous system of preterm neonates. Its isolation from tracheal aspirates is possible in the course of Hyaline Membrane Disease (HMD) (3-5).

One of the most important issues is transmission of infections caused by these organisms from mothers to the fetus in uterus or during delivery. Genital mycoplasmas can induce spontaneous abortions, chorioamnionitis, congenital pneumonia, chronic pulmonary diseases and meningitis in neonates or sometimes can lead to preterm or low birth weight infants. Also these species can lead to preterm labor (6, 7).

M. hominis and *U. urealyticum* can produce a wide variety of infectious diseases in children (3-7) and their isolation in medical laboratories is difficult due to complex nutritional requirements and their fastidious nature. However there are several studies on the prevalence of *M. hominis* and *U. urealyticum* in women in Iran; but there is not precise data about magnitude and amount of their colonization in pregnant women and their neonates (8). In some studies, it is estimated that *ureaplasma* species is present in about 40 to 80% of sexually mature women and colonization rate of *M. hominis* is reported to be 21 to 53%. This colonization is dependent on some factors such as socioeconomic status, sexual activity and age (9).

In this study, we intended to determine the colonization rate of *M. hominis* and *U. urealyticum* in pregnant women with preterm labor. A comparison was also made with another group who had normal gestational age and full term pregnancy. Therefore we used tracheal aspirates from newborns of these mothers and used PCR for detecting these organisms.

MATERIALS AND METHODS

This prospective study was performed on 165 newborns and their mothers who referred to Rasoul Akram hospital for their labor work ups from November 2010 to December 2011. The gestational age of the preterm babies at birth was assessed by the date of the maternal last menstrual period and physical examination of the newborns by Dubowitz score. During labor phase, gestational age less than 37 weeks was considered preterm; equal and more than 37 weeks was labeled as term. After delivery, a specimen was taken by a cotton swab from tracheal tube or pharynx of each newborn (considered as tracheal aspirate) and also specimen was taken from the vagina of their mothers. Multiplex PCR system (Seeplex, Seegene Company, Germany) was used to detect *M. hominis* and *U. Urealyticum* which holds a new concept oligo technology "Dual priming Oligonucleotide (DPO ™) technology. For DNA isolation we added 75ul (25mM NaOH / 0.2 mM EDTA) to the specimen. Thereafter we placed specimen in thermocycler at 98ºC for 1 hour, then reduced the temperature to 15°C. After addition of 75 ul of 40 mM Tris HCl (pH 5.5), the solution was centrifuged at 4000rpm for 3 minutes. Finally an aliquot for PCR (2 ul of a 1:100 dilution/reaction) was taken. Pre-PCR products were stored at -20˚C until use (10, 11)

The statistical tests applied in this study were the Chi square test and t-test. All significant results were based on the value of $p<0.05$.

RESULTS

The mean age of pregnant women was 25.4 ± 3.2year ($P <0.05$). The youngest woman was 18 year old and the oldest one was 38 year old. The mean gestational age was 38.2 ± 2.3 weeks. According

Table 2. Vaginal colonization of U. urealyticum and M. hominis in pregnant women at delivery

	M. hominis		*U. urealyticum*	
Gestational status	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Term	18 (13)	119 (87)	21 (15)	116 (85)
Preterm	7 (25)	21 (75)	4 (14)	24 (86)
Total	25 (15)	140 (85)	25 (15)	140 (85)

Table 3. Nasotracheal colonization of *U. urealyticum* and *M. hominis* in neonates at delivery

	M. homins		*U. urealyticum*	
Gestational status	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Term	12 (8.8)	125 (91.2)	17 (12.4)	120 (87.6)
Preterm	3 (10.7)	25 (89.3)	1(3.6)	27 (96.4)
Total	15 (9)	150 (91)	18 (11)	147 (89)

Table 4. Isolation of *U.urealyticum* and *M. hominis* from nasotracheal secretions of neonates regarding gender

	M. hominis		*U. urealyticum*	
Gender	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Male	6 (8)	68 (92)	11 (15)	63 (85)
Female	9 (10)	82 (90)	7 (7.7)	84 (92.3)
Total	15 (9)	150 (91)	18 (11)	147 (89)

Table 5. Isolation of U. *urealyticum* and *M. hominis* from nasotracheal secretions of neonates regarding type of delivery

	M. hominis		*U. urealyticum*	
Type of delivery	Positive (%)	Negative (%)	Positive (%)	Negative (%)
NVD	12 (8.6)	128 (91.4)	14 (10)	126 (90)
C/S	3 (12)	22 (88)	4 (16)	21 (84)
Total	15 (9)	150 (91)	18 (11)	147 (89)

NVD = Normal Vaginal Delivery; C/S = Cesarean Section

to definitions, 137 (83%) infants were term and 28 (17%) were preterm. 140 pregnant women had normal vaginal delivery (84.8%) and 25 pregnant (15.2%) undergone cesarean section. PCR results from the vagina specimens are depicted in Table 2.

Altogether PCR results of these two bacteria from tracheal secretions were negative in 132 (80%) neonates but positive in 33 (20%). Colonization distribution with these two bacteria was different in preterm and term deliveries. Positive PCR in term deliveries were 21.2% (29 neonates) and in preterm group was 14.3% (4 neonates). Also among 33 neonates whose tracheal PCR results for both bacteria were positive, 30 neonates (91%) were delivered naturally (NVD) and 3 neonates (9%) were born by cesarean section ($P > 0.05$). This relationship is similarly seen amongst neonates with negative PCR; 110 neonates (83%) were born by normal vaginal delivery and 22 neonates (17%) were born via cesarean section. Amongst these 33 positive PCR, 18 (54.5%) were positive for *U. urealyticum* and 15 (45.5%) cases were positive for *M. hominis.* The overall transmission from mothers to their respective neonate was 72% (18/25) for *U. urealyticum* and 60% (15/25) for *M. hominis.* There was no significant difference in the rate of transmission to term and preterm neonates. The mean birth weight was also not significantly different in babies with and without *U. urealyticum* (P=0.47) and *M. hominis* (P=0.31).

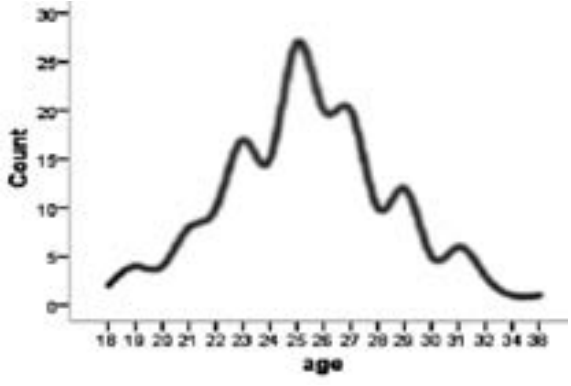

Fig. 1. Age distribution of pregnant women participated in this study

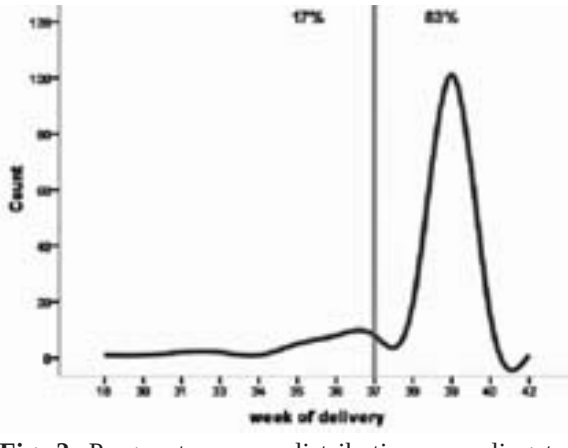

Fig. 2. Pregnant women distribution according to gestational week

DISCUSSION

In the present study, PCR results of *M. hominis* and *U. urealyticum* from tracheal aspirates of neonates were positive in 33 neonates (20%). Average age of infected mothers was 25.39 year which was not different with the average age of non infected mothers (25.64 year). There was not any statistically significant relation between types of delivery in two groups of mothers (term vs. preterm delivery). There was not a statistically significant relation between neonates with positive PCR results in term and preterm groups.

Colonization with *M. hominis* and *U. urealyticum* were not frequent in the lower genital tract of pregnant women; however transmission of these organisms to their neonates was still high. Also transmission was not affected by gestational age or birth weight. Two preterm neonates colonized with *U. urealyticum*, developed symptoms of respiratory disease during eight weeks postpartum. In healthy term babies, most mycoplasma colonization appeared to be transient and without causing any complication, but in preterm infants, both *U. urealyticum* and *M. hominis* were established causes of perinatal morbidity and mortality. Pneumonia is one of the commonest presentations of neonatal ureaplasma disease but diagnosis is difficult due to The high rate of colonization in the respiratory tract in majority of healthy individuals (9, 12). Therefore isolation of these organisms from nasopharyngeal secretions is not solely indicative of invasive lung disease even in symptomatic babies. However a positive PCR should be explained along with other evidence of disease like chest X-ray findings, WBC counts and a specific IgM response. Endotracheal secretions are more reliable specimens for PCR. Although routine screening for *U. urealyticum* is not necessary, it has been recommended that endotracheal secretions should be cultured or evaluated by PCR in preterm babies weighing less than 1250 grams, who have signs of respiratory distress, soon after birth or when they are not responding to beta-lactam therapy. A therapeutic trial with erythromycin is often used when the diagnosis of *U. urealyticum* infection is in doubt (13, 14).

Several studies have been performed to determine the prevalence of genital mycoplasmas and ureaplasma species in Iran. Using PCR on specimens from genitourinary tract of 377 patients, Najar Peerayeh *et.al*, found that 116 (30.7%) patients were positive for *U. urealyticum*, *M. hominis* or both organisms. Of these patients, 60 (51.7%) and 31 (26.7%) were positive for *U. urealyticum* and *M. hominis*, respectively and 25 (21.5%) were positive for both organisms (15). Their result indicates higher amount of colonization with these organisms in comparison to our study.

In a study by DA Silva and colleagues on 108 neonates with very low birth weight (VLBW), they prepared nasopharyngeal and endotracheal aspirates and cultured them. Then they used PCR in order to find out the relationship between *U. urealyticum*, *Chlamydia trachomatis* and development of bronchopulmonary dysplasia (BPD). Ureaplasma was detected in 40 neonates (37%) by culture and in 49 neonates (45%) by PCR. The presence of Ureaplasma was not significant in regard to development of BPD (16).

In another study by Chua *et.al*, 60 mothers and

baby pairs were examined respectively for cervical and nasopharyngeal colonization of *U. urealyticum* and *M. hominis.* Cervical colonization among mothers was 56.7% for *U. urealyticum* and 17.7% for *M. hominis*; also in another study by Chua *et.al*, the transmission rate to their infants was 88.2% and 30% for *U. urealyticum* and *M. hominis* respectively (13, 17). In our study, transmission rate of *M. hominis* and *U. urealyticum* from mothers to their neonates was 60% and 72%, respectively. In comparison to these studies, transmission of *M. hominis* to offspring is slightly higher in our findings.

According to studies on the prevalence and transmission of *U. urealyticum* and *M. hominis*, it has been found that these organisms can be transmitted from an infected female to the fetus or neonate by at least three different ways. First by an ascending intrauterine infection; in this way organisms can reach the amniotic sac. They will multiply and migrate to the fetal lung. This way of transmission mostly occurs early in pregnancy. Second route is hematogenous pathway, through placental infection by the involvement of the umbilical vessels. The organisms can be detected on maternal and umbilical cord blood samples at the time of delivery (18, 19). Important consequences of intrauterine infection with these pathogens are disseminated fetal organ involvement, chorioamnionitis and congenital pneumonia (9). The third and final route is through an infected maternal birth canal which can result in colonization of the skin, mucosal membranes and respiratory tract (20).

Screening of mothers at early pregnancy may reduce the rate of intrauterine infection of these organisms and therefore the rate of transmission to the fetus. This can prevent the serious complications such as disseminated organ involvement in fetus. Moreover by detection of these organisms in birth canal, it is possible to reduce the transmission rate at delivery by treating mothers or using cesarean section as an alternative method. However these proceedings need further evaluations and assessment of possible modalities for reducing transmission rate.

In conclusion, *M. hominis* and *U. urealyticum* are important pathogenic agents causing complications and morbidities in pregnant women as well as pneumonia, bacteremia, and meningitis in newborns. Therefore evaluation of women before planning the pregnancy is an important issue and highly recommended.

REFERENCES

1. Larsen B, Monif GR. Understanding the bacterial flora of the female genital tract. *Clin Infect Dis* 2001; 32: 69-77.
2. Patil M. Assessing tubal damage. *J Hum Reprod Sci* 2009; 2:2-11.
3. Cedillo-Ramírez L, Gil C, Zago I, Yáñez A, Giono S. Association of *Mycoplasma hominis* and *Ureaplasma urealyticum* with some indicators of nonspecific vaginitis. *Rev Latinoam Microbiol* 2000; 42:1-6.
4. Waites KB, Schelonka RL, Xiao L, Grigsby PL, Novy MJ. Congenital and opportunistic infections: Ureaplasma species and *Mycoplasma hominis. Semin Fetal Neonatal Med* 2009; 14:190-199.
5. Razin S, Yogev D, Naot Y. Molecular biology and pathogenicity of mycoplasmas. *Microbiol Mol Biol Rev* 1998; 62:1094-1156.
6. Taylor-Robinson D, Lamont RF. Mycoplasmas in pregnancy. *BJOG* 2011; 118:164-174.
7. Larsen B, Hwang J. *Mycoplasma, Ureaplasma*, and adverse pregnancy outcomes: a fresh look. *Infect Dis Obstet Gynecol* 2010; pii: 521921. doi: 10.1155/2010/521921. 2010 Jul 12
8. Cassell GH, Waites KB, Watson HL, Crouse DT, Harasawa R. *Ureaplasma urealyticum* intrauterine infection: role in prematurity and disease in newborns. *Clin Microbiol Rev* 1993; 6:69-87.
9. Waites KB, Katz B, Schelonka RL. Mycoplasmas and Ureaplasmas as Neonatal Pathogens. *Clin Microbiol Rev* 2005; 18:757-789.
10. van Kuppeveld FJ, van der Logt JT, Angulo AF, van Zoest MJ, Quint WG, Niesters HG, *et al.* Genus and species-specific identification of Mycoplasmas by 16S rRNA amplification. *Appl Environ Microbiol* 1992; 52:2606-2615.
11. Blanchard A, Hentschel J, Duffy L, Baldus K, Cassell GH. Detection of *Ureaplasma urealyticum* by polymerase chain reaction in the urogenital tract of adults, in amniotic fluid, and in the respiratory tract of newborns. *Clin Infect Dis* 1993; 17: 48-53.
12. Jeffery HE, MPH, Lahra MM (2007). The impact of infection during pregnancy on the mother and baby. In: *Fetal and Neonatal Pathology* Ed, Jean W. Keeling, T. Yee Khong. Springerl Verlag, London , UK, pp. 379-423.
13. Chua KB, Ngeow YF, Lim CT, Ng KB, Chye JK. Colonization and transmission of *Ureaplasma Urealyticum* and *Mycoplasma* hominis from mothers to full and preterm babies by normal vaginal delivery. *Med J Malaysia* 1999; 54:242-246.
14. Pandey A, Dhawan B, Gupta V, Chaudhry R, Deorari AK. Clinical significance of airways colonization with *Ureaplasma urealyticum* in premature (<34 wk) neonates. *Indian J Med Res* 2007; 125:679-684.
15. Najar Peerayeh S, Sattari M. Detection of *Ureaplasma urealyticum* and *Mycoplasma* hominis in endocervical specimens from infertile women by polymerase chain reaction. *Middle East Fertility Society Jjournal* 2006; 11:104-108.

16. Da Silva O, Gregson D, Hammerberg O. Role of *Ureaplasma urealyticum* and *Chlamydia trachomatis* in development of bronchopulmonary dysplasia in very low birth weight infants. *Pediatr Infect Dis J* 1997; 16:364-369.
17. Chua KB, Ngeow YF, Ng KB, Chye JK, Lim CT. *Ureaplasma urealyticum* and *Mycoplasma hominis* isolation from cervical secretions of pregnant women and nasopharyngeal secretions of their babies at delivery. *Singapore Med J* 1998; 39:300-302.
18. Waites KB, Crouse DT, Cassell GH. Systemic neonatal infection due to *Ureaplasma urealyticum*. *Clin Infect Dis* 1993; 17:131-135.
19. Cassell GH, Davis RO, Waites KB, Brown MB, Marriott PA, Stagno S, Davis JK. Isolation of *Mycoplasma hominis* and *Ureaplasma urealyticum* from amniotic fluid at 16-20 weeks of gestation: potential effect on outcome of pregnancy. *Sex Transm Dis* 1983; 10:294-302.
20. Cassell GH, Waites KB, Crouse DT, Rudd PT, Canupp KC, Stagno S, Cutter GR. Association of *Ureaplasma urealyticum* infection of the lower respiratory tract with chronic lung disease and death in very-low-birth-weight infants. *Lancet* 1988; 2(8605):240-245.