**Mycoplasma hominis** Infection in Spontaneous Abortions in Thrace Population: Detection by PCR

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**Abstract**

Objective: The main purpose of this study was the detection of the bacterial strain *Mycoplasma hominis*, which is one of the 14 Mycoplasma species, in spontaneous abortions.

Methods: Placental tissues from 59 miscarriages of the first and second trimester from different areas of Thrace were used. DNA was extracted using a specific kit and the presence of the *Mycoplasma hominis* strain was detected by PCR.

Results: Among the samples that were examined 2 were proven to be infected by *Mycoplasma hominis*.

Conclusion: The results of our research imply that a small percentage (~3.6%) of spontaneous abortions could be due to the presence of *Mycoplasma hominis*. PCR is a reliable, sensitive and easy method for the determination of this type of infection and could be applied in the clinical routine.

**Keywords:** *Mycoplasma hominis*; PCR; Placenta; Miscarriage

**Introduction**

*Mycoplasma hominis* is a prokaryotic microorganism with stable morphology. It is a member of Mollicutes ("soft skin") group, bacteria which are characterized by the absence of a cell wall. The Mollicutes (about 200 species) are sited in the bacterial group of Gram-positive bacteria.

A remarkable characteristic of Mycoplasmas is the tiny cell size (0.2 to 0.3 micrometers) and genome size (0.6 to 1.4 Mb). Mycoplasmas can be considered as a minimal self-replicating cell model as their genome has less than 1000 genes [1,2]. In contrast to viruses, Mycoplasmas have both DNA and RNA. Their cytoplasm doesn't have an endoplasmic reticulum or a mesosome. However, they do have ribosomes 70 S and nuclear substance.

*Mycoplasma hominis* was first isolated from a human Bartholin's gland abscess in 1937 and was named Pleuro-Pneumonia-Like-Organisms (PPLO) [3]. Because of the fact that Mycoplasmas don't have a cell wall, they don't compose peptidoglycans. Hence, they are resistant to penicillin and other antibiotics that have an effect on the structure of the cell wall. The Mollicutes (about 200 species) are sited in the bacterial group of Gram-positive bacteria.

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Mycoplasmas can be transmitted through direct contact between hosts, downward from mother to child (either during labor or through the placental membranes) and through transplanted tissues, as well as it can infect the amniotic sac early in gestation [8,9].

It is known that *Mycoplasma hominis* is associated with harmful effects on women's reproductive health, as recurrent spontaneous abortion [10] and with pregnancy complications, as ectopic pregnancy, preterm birth, preterm prelabour rupture of the membranes (PPROM), low birthweight and late miscarriage [10,11], whereas the positive correlation of *M. hominis* and infertility is still unclear. In addition, neonatal complications can be caused by maternal infection as lung diseases, meningitis and septicaemia [12].

Many researchers have found evidence indicating that *M. hominis* infection of the mother can cause reduced gestational age resulting in preterm birth and can also decrease neonatal length and weight. This supports the hypothesis that this specific microorganism has an adverse effect in many aspects of the pregnancy [13,14].

Bacterial vaginosis was also positively associated with early pregnancy loss. However due to the great number of bacteria present in this condition, researchers were unable to identify each specific pathogenetic mechanism involved. *M. hominis* is considered to be among the infections that carry the highest risk of causing recurrent spontaneous abortions [10]. In the present study, we studied the presence of *Mycoplasma hominis* infection in placental tissues coming from spontaneous abortions by PCR.

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Materials and Methods

Embryonic samples

Tissues from 59 miscarriages during the late first and the second trimester of the pregnancy were studied in the laboratory of Histology-Embryology School of Medicine, Democritus University of Thrace in Alexandroupolis. Tissues were fixed immediately in 10% buffered formalin.

Sample preparation

Tissues were placed in special biocassettes, were dehydrated in histokinette and then were embedded in paraffin.

Sample deparaffinization

Deparaffinization of the tissues was performed using the NucleoSpin® Tissue, Cat.No 74095210 kits (MACHEREY-NAGEL, Düren, North rhine-Westphalia, Germany) according to the manufacturer’s instructions. From every tissue, 3-4 paraffin microtome sections of 20 μm were taken and were placed in 1.5 ml eppendorf tubes which were then placed in a bath for 10 min at 56°C. Excess paraffin was removed from the bottom of the tubes, 1 ml of xylene was added in every tube and samples were shaken to detach the tissue from the tube walls. Tubes were left for 30 min in room temperature were centrifuged for 3 min at 11000 g and the pellet was collected. The procedure was repeated. 1 ml of ethanol was added in each tube, shaken and left for 30 min in room temperature. After centrifugation for 3 min at 11000 g the pellet was collected. The procedure from the step with ethanol was repeated. Ethanol was left to evaporate, then 180 μl of lysis buffer and 25 μl of proteinase K were added and mixed. Finally, samples were incubated overnight at 56°C.

DNA isolation

Total DNA was isolated by using the NucleoSpin® Tissue, Cat.No 74095210 kit (MACHEREY-NAGEL, Düren, North rhine-Westphalia, Germany) according to the manufacturer’s instructions.

Recently molecular methods like PCR were used to detect Mycoplasmas in several tissues [15,16]. In our case, PCR was performed to amplify the DNA sequence of the bacterium (Figure 1: Genbank accession AJ002269). We used the M. hominis, 16S rRNA, rrnB operon primer set plus positive control, 311 bp kit Cat.No SP-10501 (Maxim Biotech Inc, Rockville, Maryland, USA). The primers included were:

5’ oligo: CCGCATGGTTCCGTTGTGAA

3’ oligo: CAAGGTACCGTCAGTCTGCAATCA

PCR products were separated by 2% w/v agarose gel electrophoresis at 100 V for 1 hour and stained with ethidium bromide. In each electrophoresis except from the samples, a positive and a negative control was included (Figure 2). After electrophoresis the gel was illuminated with an ultraviolet lamp to view the DNA bands and pictures were taken by a digital camera.

Results

The results from PCR assays for the detection of Mycoplasma hominis are shown in Figure 2. A band of 311 bp in size indicates the presence of the target sequence. Among the 59 placental samples 33 were from the first trimester and the rest were from the second trimester of the pregnancy. Among each of these groups one specimens was found positive for M. hominis infection. So, from the 59 samples tested, two (3.6%) were found positive for Mycoplasma hominis and the other 57 were negative.

No PCR product could be detected in the negative control samples using water instead of the DNA template.

In Figure 2, samples 3-6, 8-9 resulted in no amplified product and thus we concluded that no Mycoplasma hominis infection was present. A DNA product of 311 bp was amplified in the positive control sample containing the DNA sequence from Mycoplasma hominis, which was included in the kit (sample 2). The same size product was also found in samples 7 and 10, indicating the presence of Mycoplasma hominis infection. All other samples were found negative and thus they are not presented in figures.
Discuss

Every five pregnancies one miscarriage occurs. The possible involvement of gestation infection has been well studied. Infections in pregnancy seem to be responsible for up 15% of early miscarriages and about 66% of late miscarriages [17]. Many different viruses have been studied over the years to prove their possible involvement in spontaneous abortions. A well-studied example is the CMV virus. Grammatikopoulou and colleagues have shown that the CMV virus may have a role in abortions even though the incidence percentage is low (1.4%) [18].

In this study, the presence of Mycoplasma hominis infection was evaluated in placental tissues, using PCR. We used placental tissues since Mycoplasma hominis can be transferred from mother to fetus through the blood stream. DNA of Mycoplasma hominis, specially the DNA sequence of 16S rRNA, rrnB operon, was detected by using a specific kit and the molecular method of PCR. In total, fifty-nine DNA samples were collected which came from placentae of 59 cases of spontaneous abortions during the first and second trimester. Among the 59 samples tested, 2 were found positive (1 coming from the late 1st trimester group and one from the 2nd trimester group) for Mycoplasma hominis and the other 57 were negative. This result implies that a percentage –3.6% of spontaneous abortions could be due to the presence of Mycoplasma hominis.

The detection of Mycoplasma hominis and other ureaplasmas by molecular-based methods, as polymerase chain reaction (PCR), is essential for the diagnosis in second-trimester amniotic fluids and can indicate that infected women are at an increased risk for pregnancy complications [4].

As it was stated, Mycoplasma hominis is a sexually transmitted microorganism that is related to a big number of pathological conditions of pregnant women. In many cases Mycoplasma hominis has been related to chorioamnionitis, fusinosis and postpartum fever but the significance of this relation is not clear [19].

During the transaction of another study, samples were collected from amniotic fluids by amniocentesis during the 16th to 20th week of gestation. Two (2) out of fifty-nine (59) samples were found positive to Mycoplasma hominis. These results indicate that Mycoplasma hominis can infect the amniotic sac early in gestation [5]. These results are in agreement with our study, as in both cases the number of samples and the percentage of infection are the same although we used samples from placentae instead of amniotic fluids. In our study, the molecular method of PCR was used which was proven to be fast, easy, sensitive and reliable for the Mycoplasma hominis detection. These results suggest that Mycoplasma hominis could be transferred from mother to fetus during the gestation and therefore be responsible for preterm labors or miscarriages.

In some cases, bacterial vaginosis and Mycoplasmas may play a causative role in spontaneous abortion and early pregnancy loss [20].

Another study of 577 pregnancies has determined the influence of Mycoplasma on peri-post-natal illness arising during the last month of gestation and during the immediate post-partum period. The incidence of contamination was 2.3% for Mycoplasma hominis [22]. This percentage is very close to the results of our clinical research (3.6% of the samples from placenta were infected with Mycoplasma hominis) [22].

Intraamniotic infections are achieved in an ascendant way and present the most common and dangerous kind of prenatal infection for the pregnant woman as well as her fetus [19,23]. After a spontaneous abortion caused by premature rupture of the fetal membranes (in infected by Mycoplasma hominis women), the fetus is usually of normal size and without malformations. Mycoplasma hominis could be isolated from the placenta and lungs of the fetus during autopsy. This is due to the fact that M. hominis mainly causes pneumonia in infants [8,24].

Overall, as stated by Leli et al. [25] the study of Mycoplasma hominis and its association with many pathogenetic conditions of the pregnancy is controversial on all aspects and the international literature seems clearly divided on this issue. Many researchers have published results that indicate no positive correlation between M. hominis infection and adverse pregnancy outcome [26,27], while others seem to state just the opposite [12,27]. This could be due to different populations included in the respective studies, different study designs, or different methods for detection. On many occasions there have been reports stating different colonization patterns for M. hominis depending on the socio-economic status and race of the population studied as well as age and sexual activity of the patients [4,6,8].

Conclusion

In conclusion, in our study Mycoplasma hominis infection was detected in 3.6% of the placentas from miscarriages in Thrace population, a percentage similar to other studies. Mycoplasma hominis is responsible for a wide spectrum of serious pathological conditions in pregnancy since it has been associated with miscarriages and spontaneous abortions. The use of placental tissue for the detection is convenient, since it is easily accessible in all miscarriages and has been proven to be reliable as it comprises a communicational bridge between mother and fetus. PCR is a fast, easy, sensitive and reliable method for the Mycoplasma hominis detection and should be introduced in laboratory routine.

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