Original Article

Detection of Mycoplasma hominis and Ureaplasma urealyticum in Blood Samples of Recurrent Pregnancy Loss in Women by Polymerase Chain Reaction.

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Summary:

Background: Genital mycoplasma are implicated in pelvic inflammatory disease, puerperal infection, septic abortion, low birth weight, nongonococcal urethritis and prostatitis as well as spontaneous abortion and infertility in women.

Objective: We aimed to find a relationship between repeated abortions of unknown etiology and caused of Mycoplasma hominis and\or Ureplasma urealyticum.

Methods: one hundred sixty cases, (15-49 years old) with history of recurrent abortion, intrauterine fetal death and\or neonatal death (after exclusion of other factors as cause abortion), and hundred women with normal pregnancy outcome with the same age were chosen as controls. M. homini and U. urealyticum were detected in blood by PCR.

Result: M. homini could be detected in 12\160 (7.5%) in women with pregnancy losses, but was not detected in control group. U. urealyticum could be detected in 64\160 (40%) in patient group and 4\100 (4%) in control group. The rapid detection of M. homini and\or U. urealyticum by PCR in pregnancy loss women could be important and necessary. The detection rate of M. homini and\or U. urealyticum in young women age (20-29 years) was higher than the others. Significant difference was observed in patients with three or four abortions compare with 2 or one abortion in addition to history of adverse outcome.

Conclusion: the role M. homini and\or U. urealyticum in the etiology of pregnancy losses was proposed.

Key words: Mycoplasma hominis, ureaplasma urealyticum, recurrent pregnancy loss, PCR.

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Introduction:

The etiologies of recurrent miscarriage are diverse and may be divided into genetic defects, such as chromosomal anomalies; maternal reproductive anatomic disease, both developmental and acquired, such as septate uterus or cervical incompetence and systemic maternal disease such as diabetes mellitus and infective factors. (1)Genital mycoplasmas represent a group of microorganisms that are commonly found in the genitourinary tract of pregnant and non-pregnant women. They have been associated with various pathological conditions and intrauterine infections, including pyelonephritis, pelvic inflammatory disease, chorioamnienitis, endometritis, and post partum fever, leading to important complications such as preterm birth, low birth weight, spontaneous abortion, still birth, premature birth, infertility, and perinatal mortality. (2,3,4,5)Infection with Mycoplasma hominis (M.hominis) or Uerplasma urealyticum (U.urealyticum) two members of the class mollicutes are considered as the possible etiological agents in the family causing pregnancy loss since the frequently produce asymptomatic infection and are not identified by routine microbiological techniques. (1)Diagnosis of mycoplasm is usually made by serological determination or in vitro isolation of the organism. However, serological procedures are often hampered by interspecies cross-reaction, while cultivation is time comsuming and hard to for some fastidious mycoplsmas. Use of mycomplasma species- specific DNA probes made it possible to discriminate between different species, this method proved to be sensitive and specific. (6,7)The present study aimed at finding the relationship between fetal losses of unknown etiology and presence of mycoplasma infection (Mycoplasma hominis and Ureplasma urealyticum) in blood samples from pregnancy losses by polymerase chain reaction (PCR) technique.

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Patients and methods:-

Subjects:- two groups of women, age ranged between (15-49 years) were included in this study. The first group was 160 women with a history of pregnancy loss, such as, repeated abortion, intrauterine fetal death and/ or neonatal death. Medical examination and family history complete physical examination, laboratory investigations were done to exclude other causes of abortion. Complete records were sought of prior pregnancies, gynecologic surgery, non-steroidal estrogen exposure in utero and findings pathological examinations. The second group (control) composed of 100 women had at least one live birth with out pregnancy wastage.

Setting :- in the family planning in AL-Elwiya hospital and

primary health center services in Bab-Almudhum.

Duration of study:- data collection continued for a period of one year starting on October 2011 and ending on October 2012.

Methods:- blood samples were collected using EDTA as anticoagulant to detect mycoplasma hominis and ureplasma urealyticum using PCR technique.

DNA extraction:- blood samples were centrifuged at 3500 rpm for 20 min, cell pellets were treated with 20 mM Tris-HCI (pH 8.0), 1mM EDTA (pH 8.0), 5% SDS with 100 Ug mL ^{1} proteinase K.DNA was extracted using phenol-chloroform-isoamyl alcohol (25:24:1). The DNA was precipitated with 0.1 volume of 2 M potassium chloride and double volumes of absolute ethanol, air dried (8). The DNA pellets were suspended in TE buffer and incubated at 37°C for 30 min. Bioron \ Germany (Real line DNA extraction 2kit).PCR amplification:- Amplification of gene sequences was performed in a total volume of 50 mL of PCR buffer (10mM Tris-HCL, 50 mM KCL, pH 9) containing 0.1% Triton X-100, 200 mm each of dATP, dTTP, dGTP and dCTP, 100 pmol of each primer and 0.5 mg of DNA. purified mycoplasmal DNA(0.5-1 ng of DNA) was used as a positive control for amplication. The amplification was carried out for 40 cycles with denaturing at 94°C and annealing at 60 °C Extension temperature was 72°C: finally, product extension was performed at 72 °C for 10 min. Bioron \ Germany (Real line Flaformat kit).

Primers used as Mycoplasmal group specific (generic) PCR were prepared according to Ossewaarde et al. (9). as follows: upstream primer GPO-3,5- GGGA GCAA ACAG GATT AGAT TAGA TACC CT-3, downstream primer MGSO, 5-TGCAC CATC TGTC ACTC TGTT AACCTC-3 (expected size was 295 bp). Primers used in Ureaplasma urealyticum were prepared as follow : U.u. F1: 5- GCTAA TACCG AATAA TAACA TC-3 and U.u. R1:5-ATGGT ACAGT CAAAC TAAAA TC3 (expected size was 311 bp).

Statistical analysis:- Chi- square (X^2) test was used for the generation of P<0.05 value.

Result:

Results of PCR using primers specific for detection of mycoplasma are present in table (1). Out of 260 subjects, 87(33%) were positive for M. homini and\or U. urealyticum of these, 83 women were in the patient group (52%) and four were in the control group (2.5%). M. homini was isolated alone from 12\160 (7.5%) patients; no M. homini was detected in controls. The frequency of U. urealyticum detected alone in the patient group was 64\160(40%) and (4\100) controls (p<0.05). seven women within the patient group (4%) were co-infected with both M. homini and U. urealyticum .

Table (1): Frequency of Mycoplasma homini and Ureaplasma urealyticum among patients and control womens.

Group	Positive Mycoplasma homini (M.h) (%)	Positive Ureaplasma urealyticum (U.u) (%)	Positive mixed M.h and U.u (%)	Total (%)
Patients (N=160)	12 (7.5)	64 (40)	7 (4)	83(52)
Control (N=100)	0 (0)	4 (4)	0 (0)	4(2.5)
Total (N=260)	12 (5)	68 (26)	7 (3)	87(33)

p<0.05

on comparing the frequency of genital mycoplasmas among the different age groups. We studied 160 female patients (pregnant loss) their ages ranged between the age of 15-49 years. The highest frequency of M. homini and U. urealyticum was seen in the age group of 20-24 years about 50% and 25% in age group 25-29 years for positive cases of M. homini and 36% were aged 25-29 years in positive cases of U. urealyticum, table (2). Significantly approximately two thirds of M. homini and U. urealyticum occurred in patients between the ages of 20 and 29 years (p<0.05).

Table (2): Frequency of Mycoplasma homini (M.h) and Ureplasma urealyticum (U.u) in different age groups.

Age range (years)	Total patient no. (%)	Positive cases		
		M.h no.(%)	U.u no.(%)	Mixed(M.h+U.u) no.(%)
15-19	8 (5)	0 (0)	1 (1.5)	1 (14)
20-24	32 (20)	6 (50)	10 (16)	4 (58)
25-29*	42 (26)	3 (25)	23 (36)	1 (14)
30-34	34 (21)	1 (8)	9 (14)	0 (0)
35-39	17 (11)	2 (17)	12 (19)	1 (14)
40-44	21 (13)	0 (0)	6 (9)	0 (0)
45-49	6 (4)	0 (0)	3 (4.5)	0 (0)
Total (%)	160 (100)	12 (100)	64 (100)	7 (100)

*p<0.05

Presence of genital mycoplasmas differ according to number of repeated abortions (table 3); such that M. homini was detected in the blood of cases suffering from three or four losses more than those with two or less pregnancy losses. Three cases out of 27 suffered from one abortion in addition to history of adverse outcome (neonatal death and\or intrauterine fetal death) were positive for M. homini (11%). Also U. urealyticum positive cases was detected from three repeated abortion (69%) more than two repeated abortions (19%). And 14 cases positive for U. urealyticum (52%) in cases suffered from one abortion in addition to history of adverse outcome. There was statistically significant diference between the frequency of M. homini and\or U. urealyticum and increase number of repeated abortion (p≤0.001).

Table (3): Frequency distribution of PCR-positive Mycoplasma homini and Ureaplasma urealyticum in relation to No. of pregnancy losses.

PCR positive bacteria	No. of pregnancy losses (160)				Total (%)
	No. of repeated abortion			Causes suffered from abortion and adverse out come (%) *	
	≥ 4 (%)	3 (%)	2 (%)		
Mycoplasma homini (M.h)	3\56 (5)	5\35 (14)	1\42 (2)	3\27 (11)	12\160 (7.5)
Ureaplasma urealyticum (U.u)	18\56 (32)	24\35 (69)	8\42 (19)	14\27 (52)	64\160 (40)
Mixed M.h and U.u	2\56 (4)	3\35 (9)	1\42 (2)	1\27 (4)	7\160 (4)

p≤0.001

* Adverse outcome still birth (intrauterine fetal death) and neonatal birth.

Spontaneous abortion at a gestation age of less than 36 weeks is shown (table 4). Of these 9(75%) cases had been colonized with M. homini and 50 (78%) with U.urealyticum in patient group. While in still birth (intrauterine fetal death) 3(25%) cases positive on M. homini and 11(17%) positive of U. urealyticum; also 3(5%) cases positive of U. urealyticum in neonatal death in patient group. Some of the patients were co-infected with M. homini and U. urealyticum (7 cases) as follows. Five cases with spontaneous abortion and 2 cases with intrauterine fetal death. The difference was statistically highly significant (p≤0.001).

Table (4): Number and percentages of pathological conditions observed in patients women and their possible association with mycoplasmas infection.

Pathological conditions	Positive cases		
	Patient group (No.=76)		
	* M.h (%)	** U.u (%)	M.h and U.u
Spontaneous abortion	9 (75)	50 (78)	5 (71)
Still birth	3 (25)	11 (17)	2 (29)
Neonatal death	0 (0)	3 (5)	0 (0)
Total (%)	12 (100)	64 (100)	7 (100)

p≤0.001

*M.h= Mycoplasma homini

** U.u= Ureaplasma urealyticum

Discussion :

The relation mycoplasma hominis and Ureplasma urealyticm and repeated pregnancy loss was observed previously by several outhors. In the present study, M.hominis was detected in blood of 12 (7.5%) women with repeated pregnancy losses, and not in normal women (control), however (40%) of pregnancy loss women and (4%)in control group were colonized with U.urealyticum. 7 (4%) where infected with both M.hominis and U.urealyticum, where as none of the controls. As result detected by PCR technique. This is alittle higher than results obtained by Elias et al. (10) who in a group of 222 women in similar age range, found U.urealyticum in (31.8 %) and M.hominis in only (3%) of the cases. Schlicht et al. (11) found ahigher prevalence of 54% for U.urealyticum. Agbakoba (12) found a prevalence of (36.7%) for genital mycoplasmas while working with Nigerian women. Zdrodowska- stefanow et al. (13) in a similar study in pregnancy losses women, showed that U.urealyticum was detected in 161 (29.8%) and M.hominis in 20 (3.7%) women.Bayoumi et al. 2006 (1) concluded that the interaction between mycoplsamas and pro-inflammatory markers, which consist primarily of the development of specific antibody and non-specific interactions with B lymphocytes or antibody which responses are important in the resistance of mycoplasmal disease in human. However, the ability of mycoplasmas to survive in their host despite vigorous responses suggests that these play a limited role in the host recovery from infection accounting for the appearance of systemic mycoplasma infection. In some cases, antibody response may contribute to the disease pathogenesis through the development to hypersensitivity responses or the deposition of immune complexes leading to auto immune reaction. Pandey et al 2005 (14) reported that majority of cases with unexplained cause of abortion are found to be associated with certain auto immune antibodies that may play a role in the immunologic failure in pregnancy and may lead to abortion. At present, the main method of detecting M.hominis and / or U.urealyticum is by culture, but the cultivation of mycoplasma infection is laborious, is time consuming, and requires specific expertise. PCR is revolutionizing the diagnosis of many infectious diseases, particularly those caused by organisms that

are difficult to cultivate. PCR is a more sensitive and reliable means of detecting M.hominis and U.urealyticum in blood and / or endocerivcal specimens; its results can be available within a day, compared with 2-5 days for culture. (6, 15)It was also observed that genital mycoplsama infection is specific to age group. The highest frequency was in the 20-29 years group with 9 (75%) with M.hominis and 33 (52%) in U.urealyticum. It is related to sexual activity, hence and sexual active group is a potential carrier. Asmilar result was obtained by zdrodowska-stefanow et al. (13) with the highest rate in the age range of 26-30 years (29.2% for U.urealyticum and 50.0 % for M.hominis). The presence of genital mycoplasmas is associated with an increased risk of developing certain pathologic conditions of pregnancy, such as spontaneous abortion, preterm labor, and low birth weight. (2). In the present study, 75% of pregnant loss women infected with M.hominis and 78% infected with U.urealyticum in spontaneous abortion. More over 3/12 (25%) of M.hominis and 11/64 (17%) of U.urealyticum in still birth. While 3/64 (5%) of U.urealyticum in neonatal death. Cases had been colonized with M.hominis and /or U.urealyticum. This discrepancy may be due to variations in socieoeconomic conditions and living standards. (2) since M.hominis and /or U.urealyticum has been found significantly associated with low socioeconomic back ground, such as poverty, number of sexual partners, and use of contraceptive drug. (16,17). A change in vaginal pH (e.g., bleeding in pregnancy, sexual intercourse, or vaginal douching) may predispose to an overgrowth of potential pathogens. (18) Although the precise role of M.hominis and / or U.urealyticum has been implicated in several complication of pregnancy and in neonatal morbidity and mortality. It may plays roles in endometritis, choriamnionitis, premature rupture of membranes, prematurity, low –birth weight infants, postpartum fever and it is important causes of pneumonia and meningitis in very low-birth –weight infants. (6)Finally, we concluded, the prevalence of M.hominis and U.urealyticum infection was significantly correlated with the etiology of pregnancy losses. And we recommend that all women with poor pregnancy outcome, before planning subsequent pregnancy, should test for the presence of bacterial infections (Mycoplasma infection)

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