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Detection the distribution of *Ureaplasma parvum* in women with recurrent miscarriage by polymerase chain reaction assay

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ABSTRACT

One hundred Seventyample were collected, including vaginal bleeding, vaginal swabs and urine. One hundred and thirty samples of women were collected with repeated miscarriage and forty samples of control. Two types of media (middle IH broth) and IH agar medium were used. A positive isolate of Mirablasma spp. In culture, the PCR probe was investigated to determine Ureaplasma parapum and subtyping to (SV1, SV3, SV6, SV14). Results showed that Ureaplasma was determined in 29.6% of the patient and 11% of control. The serovar3 isolate were isolated by the use of the PCR test, and results revealed that serovar3 was more isolated in the rate (42.8%), while serovar1 (28.5%), serovar6 (14.2%) and serovar14 (14.2%) in the patient but only serovar1 control was isolated at rate (11%). The findings suggest that the Ureaplasma parvum infection may be an important archaeological factor for repeated miscarriage and serovar3 was the most frequent serovar detected in the current study.

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INTRODUCTION

Repeated abortion is the loss of more than three consecutive pregnancies that end with pregnancy by aborting a fetus or fetus before it can survive ectopic (Grime and Stuart, 2010; Urzula *et al.,* 2014). The World Health Organization (WHO) has identified about 56 million recurrent abortions before the 24th week of pregnancy, which occur every year in the unwarranted world (Capoccia *et al.,* 2013; Redelinghuy *et al.,* 2015). Any acute genital tract infection that leads to bacteremia or viremia can cause miscarriage. Ureaplasma parapum may be an important pathogen that may affect pregnancy outcome and was first considered to be severe neonatal health when reports of postpartum endometritis with septicemia, choroiditis (Leitich and Kis, 2007; Ning *et al.,* 2014). Since then, many clinical studies have shown that organism play the role of the factor responsible for invasive gonadal infection, early delivery, spontaneous abortion, stillbirth, and chronic lung disease. (Zhang *et al.,* 2014). Despite the passage of more than thirty years of study inside and outside Iraq, many of the virulence factor and clinical significance of Ureaplasma parvum genitalia are still not justified for any reason. These include 1. The high rate of the organism in healthy person 2. The simple design of many previous research studies, which attempted to relate more to the presence of Ureaplasma paravum in the lower genital tract to pathology in the upper tractor in the offspring (Huang *et al.,* 2015; Wetmore *et al.,* 2011).

Ureaplasma parapum is located in the placenta and endometrium, with infection. The birth of a dead fetus, abortion, premature birth, and le than the normal weight of the infant. Bacterial coagulation, in selenium, in the second trimester of pregnancy, may be the cause, chimonionitis, (Capoccia *et al.,* 2013). Uranplasma parapum, in the blood, is found from mother, who suffers from the problem, with high temperature, postpartum, this infection, can be transmitted to about 40% of children born, to

the mother, with this infection, if Mother, it has (Redelinghuy *et al.,* 2015). Another study found that Ureaplasma parvum is dominant in patients with pelvic, inflammatory, and disease, as well as in women who have had miscarriage and appear to have more negative effects on pregnancy and outcome in relation to childbirth, delivery, pregnancy and preterm birth of U. urealyticum, Ureaplasma parvum was found to be more frequently isolated from patients with a history of repeated miscarriage than that of normal pregnant women (Urzula *et al.,* 2015). The main objective, from this, study, to investigate the occurrence, Ureaplasma parvum, in women, with repeated miscarriage and to determine the distribution (SV1, SV3, SV6, SV14). In patients with repeated miscarriage by PCR test.

MATERIALS AND METHOD

The Bacterial Isolates

One hundred seventy samples were collected, vaginal, bleeding, vaginal swab, and urine. 130 sample of women were collected with repeated miscarriage and 40 samples of dominant women. All sample were cultured in IH medium Al-(Azawiy, 2013). After culture examination the bacterial growth was done using microscopic light colonies, directly, colonies, from Ureaplasma spp. Dark brown due to the accumulation of magnesium, oxide, inside and outside.

Molecular Experiments

Molecular experiments included the extraction of Ureaplasmas DNA by using Reagents Genomics DNA Kit (Geneaid, USA) and amplification of U.parvum DNA. PCR identification of U.parvum was made according to Kongs *et al.,* (2000). And master mix kits (BioNeer/Korea). PCR was performed with primer specifics for the highly conserved region in the 5' end of (MBA) gene of U.parvum. Primer for diagnosis U.parvum UM-57/UMA222as shown in (Table1). Primer for detection U.parvum serovar UM3S/UMA26s, UM14S/UMA314As, UM-83s/UMA1As, and UM-54s/UMA269s (BioNeer/Korea) as shown in (Table2). were used for subtypings of U.parvum to amplify the repetitive of the (MBA) gene of U.parvum serovar.

Polymerase Chain Reaction Technique

The 20ul amplification reaction mixture contained 10pmol of each primer, 5ul of DNAs template and PCR water added to 20ul. For identification Ureaplasma parvum the PCR condition used were Initials denaturation at 95C for 5 min, cyclists denaturation at 95C for 30 secs, annealings at 58C for 30 sec, extension at 72C for 1 min for 40 cycle and finals extension at 72C for 5 min in a thermocycler. PCR positive for U.parvum were further subtyped into serovar as described in (table2). Briefly, the PCR condition used were Initials denaturation at 95C for 5 min, denaturation at 95C for 30sec, annealings at 55-62C for 30sec, extension at 72C for 1 min for 40 cycles. PCR products (10ul) were analysed by electrophoresis on 2% agarose gels which were stained with 0.5mg/ml of ethidium bromide. A visible band of the appropriate size on UV transillumination was considered a positive result.

Statistical Analysis

The data was analysed using SPS statistics software version 20. For comparison of the qualitative variable. Using (P<0.05) & odd ratios. association between U.parvum infection and recurrent abortion was statistically significant.

RESULTS AND DISCUSSION

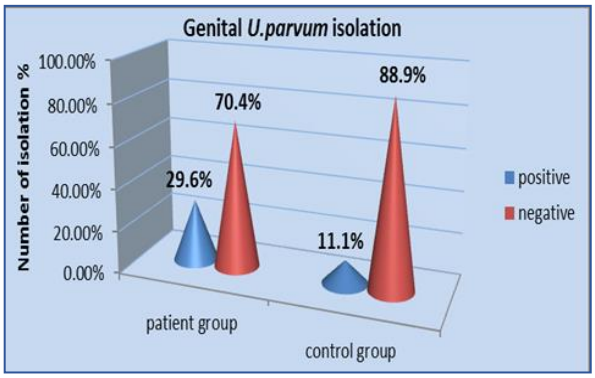

Figure 1: Prevalence of Ureaplasma parvum among a patient group and the control group

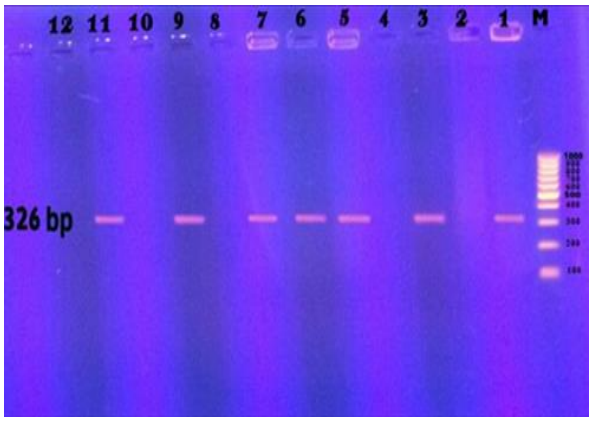

Figure 2: Ethidium bromide stained agarose gel showing PCR amplification products with (326bp) primer for U.parvum

The results showed the *U.parvum* isolated in rate (29.66%) from women with recurrent miscarriage and (111%) from controls as shown in (figure1). {p<0.05 appeared significants highly}. The results revealed positive isolate by using UM-57/UMA222s primer as shown in (figure2). The negative isolate may be due to that *Ureaplasmas* are divided into two spp. These are *U. parvum* and So the negative results may be *U.urealyticum*, these two spp. Can't identify by phenotypic and only identified by genotypic (Kong *et al.,* 2000).

Table 1: PCR primer employed in the detection of Ureaplasma parvum

organism	Primer (F) (R)	sequence (5'- 3')	size of the amplified product (bp)	Target gene
U.parvum	UM-57 UMA222	F (AA ATC TTA GTG TTC ATA TTT AC) R (GTA AGT GGA TTA AAT TCA ATG 222)	326	5 Ends of MBA gene and upstream regions

MBA gene. Adapted with permission from. (Knox *et al.,* 2013; Ning *et al.,* 2014)

Table 2: PCR Primer employed for subtyping of U. parvum into erovar

Organisms	Primer (F) / (R)	sequence (5'- 3')	size of the amplified product (bp)	Target gene
V 1	UM-83 UMA1A	F (TTACT GTA GAA ATT ATG TAA GAT TGC) R (TTT CTT TTG GTT CTT CAG TTT TTG AAG)	578	MBA
V 3	UM3 UMA269	F (TTA CTG TAG AAA TTA TGT AAG ATT ACC) R (AA CTA AAT GAC CTT TTT CAA GTG TAC)	400	MBA
V 6	UM-54 UMA269	F (AAT CTT AGT GTT CAT ATT TTT TAC TAG) R (ACCA AAT GAC CTT TTG TAA CTA GAT)	370	MBA
V 14	UM14 UMA314A	F (AAT TAC TGT AGA AAT TAT GTA AGA TTA AT) R (GTT GTT CTT TAC CTG GTT GTG TAG)	572	MBA

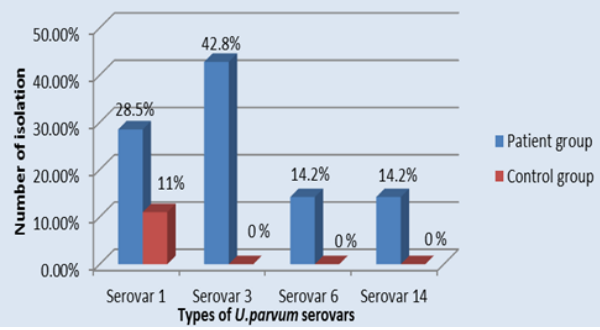

Figure 3: Distribution of Ureaplasma parvum serovar among the patient group and the control group

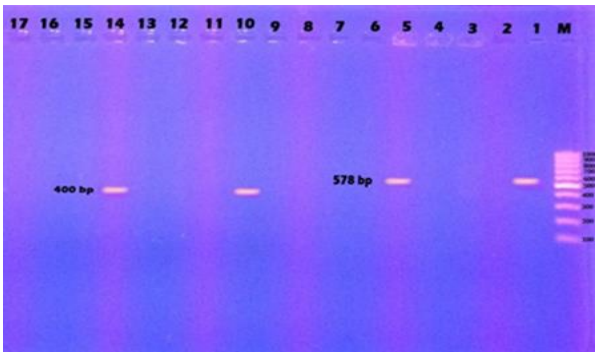

Figure 4: Results of PCR amplification for identification of serovar 1 (578 bp) and serovar 3 (400bp)

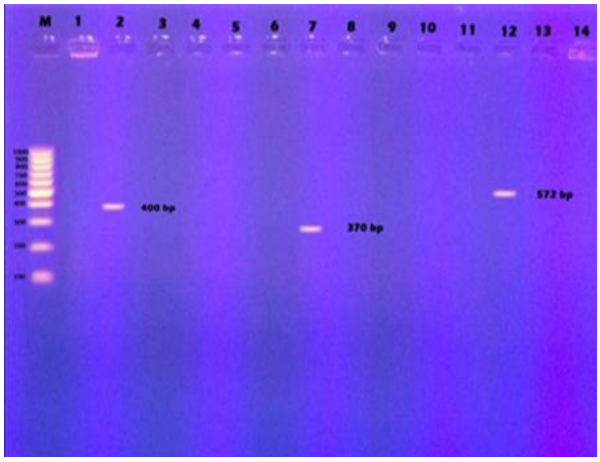

Figure 5: Results of PCR amplification for identification of serovar 3 (400 bp), serovar 6 (370 bp) and serovar 14 (572 bp)

So the negatives results may be *U.urealyticums* rathers than *U.parvums*.and the results appeared to be attributable to higher proportions of women with recurrent abortions in whom *U.parvums* were found the reasons for this result isuncertains, but it could is dues to hormonal effects which could increase *U.parvum* counts and thus the likelihoods of detections during pregnancy. Others studies were isolated *Ureaplasmas parvums* in rates (20%) from women with recurrent abortions in chinas by using PCRs technique (Drawn *et al.,* 2012). While *Ureaplasmas parvum* was isolated in rates (25%).

from women with symptoms of urethral, cervicals discharges, genitals pruritis, dysuria in Indias. However, some other studies detected these organisms in highs rates approximately (79%) from pregnant women and women with sexually transmitted diseases in Australia (Kong *et al.,* 2000).

Ureaplasmas parvums positives isolates were furthers subtyped into these serovars (1, 3, 6, 14) the results revealed *U.parvum*s (biovars2) serovars3 was predominant among women with recurrent abortions. As shown in (figure 3), (figure 4), (figure 5). *U.parvums* serovars 3 was isolated in rate (42.8s%) the most frequents isolates in women with recurrent abortions followed by serovars 1 in rate (28.5%) while serovars 6 in rate (14.2%) and serovar 14 in rate (14.2%) in patients groups, however in controls groups *U.parvums* was isolated only serovars 1 in rate (11%). Among the different serovars of *U.parvum*s, serovars 3 was the most frequent serovars detected in patients groups. Therefore *U.parvums* (biovars 2) serovars 3 was predominant among women with recurrent abortions and suggest the *U.Parvums* serovar3 there is evidence that it may play a roles in recurrent abortions and prematurity also mays be related to intraamniotic inflammatory responses to *U.parvum*s and that this is related not only to recurrent abortions but also to early onsets sepsis in the baby. Through the differences in detections rates of the different serovars of *U.parvums* was statistically significant, predominances of serovars 3 were consistent with previous reports (Kong *et al.,* 1999). Another study detected *U.parvum* serovars 3 is the most prevalent serovars detected in reproductives humans (Knox *et al.,* 2013). Another study isolated the completes genomes sequences of *U.parvum* serovars3, clinical strains SV3F4s, isolated from a Japanese patient who had an infectious abortion during the 13th gestational weeks in her previous pregnancy. Also Urszulas, *et al.,* (2014). Isolated *U.parvums* serovars 3/14 in 86% of women with symptomatic genitals tracts infections. It is possibles that the combinations of variables serovars specifics genes of *Ureaplasma*s with generally Knowns virulences factors determiness the developments of pathological processes on the mucosal surfaces of the human genitals tracts. *Statistical analysis include (P –value = 0.001) the P-values <0.05 showeds highlys significants betweens patients and controlss groups accordings to isolations of *U.parvums* serovarss.

CONCLUSION

Thee results indicate that demonstrated a correlation between blood group antigen and susceptibility to Ureaplasma parvum infection.

Conflicts of interests: There are no conflicts of interests.

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