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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 seventy specimen included vaginal bleeding , vaginal swab and urine , were collected One hundred thirty specimen from women with recurrent miscarriage and fourtyspecimen from control. Through the research two types of media were used (IH broth medium) and (IH agar medium). positive isolates of *Ureaplasma spp*. in culture were investigated by PCR assay to identification of *Ureaplasma parvum* and subtyping to (SV1, SV3, SV6, SV14). The results showed the *Ureaplasma parvum* was identified in (29.6%) from patient and (11%) from control. *Ureaplasma parvum* isolates were further subtyped by using PCR assay, the results revealed the serovar3 was the most repeater isolate in rate (42.8%), while serovar1(28.5%), serovar6 (14.2%) and serovar14 (14.2%) in patient but in control only serovar1 was isolated in rate (11%). These results indicate that *Ureaplasma parvum* infection may be an important etiologic agent of recurrent miscarriage and serovar3 was the most repeater serovar detected in present study.

Keywords: *Ureaplasma parvum* , serovars, IH medium ,recurrent abortion , PCR, subtyping..

1-Introduction

Recurrent abortion is the loss of more than three consecutive pregnancies ending of pregnancy by miscarriage a fetus or embryo before it can survive outside the uterus [1],[3]. And explained the WHO around 56 million recurrent abortions before the twenty four week of pregnancy occur each year in the world unexplained [4],[5]. Any severe genital tract infections that leads to bacteremia or viremia can cause abortion. *Ureaplasma parvum* could be important pathogens that may affect pregnancy outcomes and the health of neonates was first given serious consideration when reports of postpartum endometritis with septicemia, chorioamnionitis [12],[14]. Since those days, numerous clinical studies explain that organisms play as agents responsible for invasive infections in neonates, premature labor, spontaneous abortion, stillbirth, and chronic lung disease of prematurity [13],[15].

Although more than thirty years of study inside and outside of Iraq, many virulence factors and clinical importance of genital *Ureaplasma parvum* are still unexplained for many of reasons. These include 1.The high rate of these organisms in healthy persons 2.simple design of many of the earlier research studies, which attempted to relate the more presence of *Ureaplasma parvum* in the lower genital tract to pathology in the upper tract or in offspring [15,16]. *Ureaplasma parvum*found in the placenta and endometrium is associated with infection the birth of a dead fetus, miscarriage,

premature delivery and lower than normal weight of infant. *Ureaplasma parvum* penetrate into amnion, in the second trimester it may cause chorioamnionitis [4]. *Ureaplasma parvum* was found in the blood of mothers who have had problems with high fever after childbirth this infection can be transmitted to about 40% of babies who were born, to a mother, with this infection if the mother has it [5]. Other study demonstrated that *Ureaplasma parvum* to be dominant in patients with pelvic inflammatory disease as well as in women who had miscarriages and it seemed to have more adverse effects on pregnancy outcome regarding birth weightgestational age and preterm birth than U.urealyticum and shown that *Ureaplasma*parvum can be isolated more frequently from patients with a history of recurrent miscarriages than from normal pregnant women [2].

The main aim of this study to investigation the occurrence, of *Ureaplasma parvum* in women, with recurrent miscarriage and to determine the distribution of (SV1, SV3, SV6, SV14). in patients with recurrent miscarriage by PCR assay.

2-Materials andmethods

2.1-The Bacterial Isolates

In total, 170 specimenincluded vaginal bleeding vaginal swab and urine were collected 130 specimen from women with recurrent abortion and 40 specimen from control women. All specimens were culture in (IH medium)[6]. After culture examination the bacterial growth by using Light microscope Colonies were investigated directly the colonies of *Ureaplasma spp.* as dark brown color due to accumulation of magnesium oxide inside and outside the colony

2.2-Molecular Experiments

Molecular experiments included the extraction of Ureaplasma DNA by using Reagent Genomic DNA Kit (Geneaid , USA) and amplification of *U.parvum* DNA. PCRs identification of *U.parvum* was done according to Kong et al [7]. and master mix kit (BioNeer /Korea). PCRs was performed with primers specific for highly conserved regions in the 5' ends of (MBA) genes of *U.parvum*. Primer for diagnosis *U.parvum*UMS-57/UMA222as shown in (Table1).Primers for detection *U.parvum* serovars UMS3S /UMA26 , UMS14S /UMA314A , UMS-83 /UMA1A , and UMS-54 /UMA269 (BioNeer /Korea) as shown in (Table 2). were used for subtyping of *U.parvum* to amplify the repetitive of the (MBA) genes of *U.parvum* serovars .

2.3-Polymerase Chain Reaction Technique

The 20ul amplification reaction mixtures contained 10pmol of eachs primers , 5ul of DNAs template and PCR waters added to 20ul. for identification *Ureaplasma parvum* the PCR condition used were Initial denaturation at 95C for 5 min, cyclic denaturation at 95C for 30 sec, annealing at 58C for 30 sec, extension at 72C for 1 min for 40 cycles and final extension at 72C for 5 min in a thermocycler . PCR positive for *U.parvum* were further subtyped into serovars as described in (table2). Briefly the PCR conditions used were Initial denaturation at 95C for 5 min, denaturation at 95C for 30sec, annealing at 55-62C for 30sec, extension at 72C for 1 min for 40 cycles . PCR products (10ul) were analyzed by electrophoresis on 2% agarose gels which were stained with 0.5mg/ml of ethidium bromide . A visible band of the appropriate sizes on UV translumination was considered a positive results.

2.4-Statistical Analysis

The data was analyzed using SPSS statistic software version 20. For comparison of qualitative variables . Using ($P<0.05$) & odd ratio . association between *U.parvum* infection and recurrent abortion was statistically significant .

Table 1 : PCR primers employed in the detection of *Ureaplasma parvum*.

organisms	Primers (F) (R)	Sequence (5'- 3')	Size of amplified product (bp)	Target genes
U.parvum	UMS-57	F (AA ATC TTA GTG TTC ATA TTT AC)	326	5 Ends of MBA genes and upstream regions
	UMA222	R (GTA AGT GGA TTA AAT TCA ATG 222)		

MBA gene. Adapted with permission from [10,11].

Table 2: PCR Primers employed for subtyping of *U. parvum* in to serovars.

Organism	Primers (F)/(R)	Sequence (5'- 3')	Size of amplified product (bp)	Target gene
SV 1	UMS-83 UMA1A	F (TTACT GTA GAA ATT ATG TAA GAT TGC) R (TTT CTT TTG GTT CTT CAG TTT TTG AAG)	578	MBA
SV 3	UMS3S UMA269	F (TTA CTG TAG AAA TTA TGT AAG ATT ACC) R (AA CTA AAT GAC CTT TTT CAA GTG TAC)	400	MBA
SV 6	UMS-54 UMA269	F (AAT CTT AGT GTT CAT ATT TTT TAC TAG) R (ACCA AAT GAC CTT TTG TAA CTA GAT)	370	MBA
SV 14	UMS14S UMA314A	F (AAT TAC TGT AGA AAT TAT GTA AGA TTA AT) R (GTT GTT CTT TAC CTG GTT GTG TAG)	572	MBA

3- Results andDiscussion

The results showed the *U.parvum* isolated in rate (29.6 %) from women with recurrent miscarriage and (11 %) from control as shown in (figure1). {$p<0.05$ appeared highly significant }.The results revealed positive isolates by using UMS-57/UMA222 primer as shown in (figure2). The negative isolates may be due to that *Ureaplasma* are divided into two spp. These are *U.parvum* and *U.urealyticum* , these two spp. Cann't identify by phenotypic and only identified by genotypic [7]. So the negative results may be *U.urealyticum* rather than *U.parvum* .and the results appeared to be attributable to a higher proportion of women with recurrent abortion in whom *U.parvum* were found the reason for this results is uncertain but it could be due to hormonal effects which could increase *U.parvum* counts and thus the likelihood of detection during pregnancy . Other studies was isolated *Ureaplasma parvum* in rate (20%) from women with recurrent abortion in china by using PCR technique [8].While *Ureaplasma parvum* was isolated in rate (25%) [9].from women with symptoms of urethral , cervical discharge , genital pruritis , dysuria in India . However , some other studies detected these organisms in high rate approximately (79%) from pregnant women and women with sexually transmitted disease in Australia [7].

Ureaplasma parvum positive isolates were further subtyped in to these serovars (1,3,6,14) the results revealed *U.parvum* (biovar 2)

serovar 3 was predominant among woman with recurrent abortion . As shown in (figure3), (figure4),(figure5). *U.parvum* serovar 3 was isolated in rate (42.8 %) the most frequent isolate in woman with recurrent abortion followed by serovar 1 in rate (28.5%) while serovar 6 in rate (14.2%) and serovar 14 in rate (14.2%) in patient group , however in control group *U.parvum* was isolated only serovar 1 in rate (11%). Among the different serovars of *U.parvum* , serovar 3 was the most frequent serovar detected in patient group . Therefore *U.parvum* (biovar 2) serovar 3 was predominant among woman with recurrent abortion and suggest the *U.Parvum* serovar3 there is evidence that it may play a role in recurrent abortion and prematurity also may be related with intraamniotic inflammatory response to *U.parvum* and that this is related not only to recurrents abortion but also to early onset sepsis in the baby . Through the difference in detection ratess of the different serovars of *U.parvum* was statistically significant , predominance of serovar 3 was consistent with previous reports [9]. Another study detected *U.parvum* serovar 3 is the most prevalent serovar detected in reproductive humans [10]. Other study isolated the complete genome sequence of *U.parvum* serovar 3, clinical strain SV3F4 , isolated from a Japanese patient who had an infectious abortion during the 13th gestational week in her previous pregnancy (11). Also Urszula , et al [2]. isolated *U.parvum* serovar 3/14 in 86% of women with symptomatic genital tract infections . It is possible that the combination of variable serovar specific genes of *Ureaplasma* with generally Known virulence factors determines the development of pathological processes on the mucosal surface of the human genital tract . *Statistical analysis include (P –value = 0.001) the P-value <0.05 showed highly significant between patient and controls group according to isolation of *U.parvum* serovars .

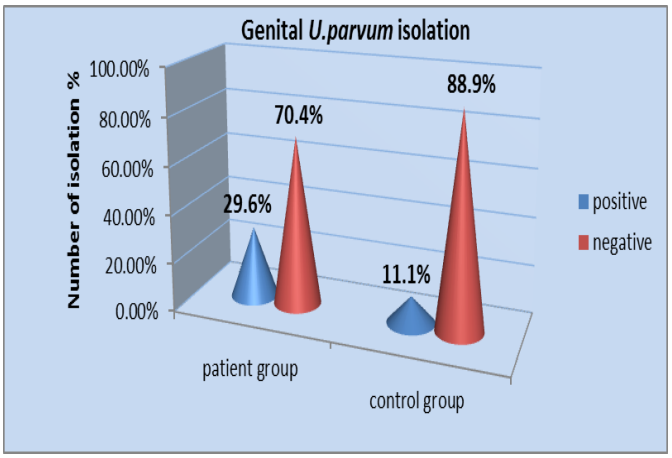

Figure-1: Prevalence of *Ureaplasma parvum* among patient group and control group .

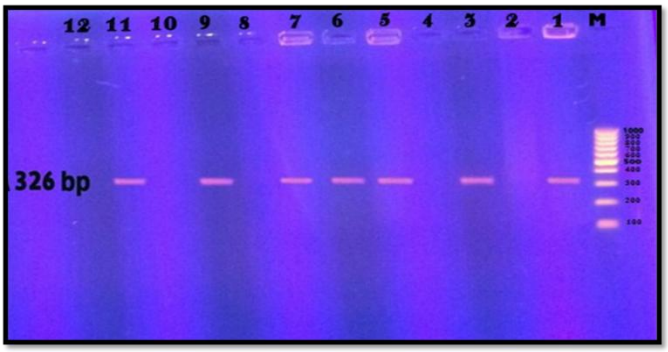

Figure -2 : Ethidium bromide stained agarose gel showing PCR amplification product with (326bp) primers for *U.parvum* .

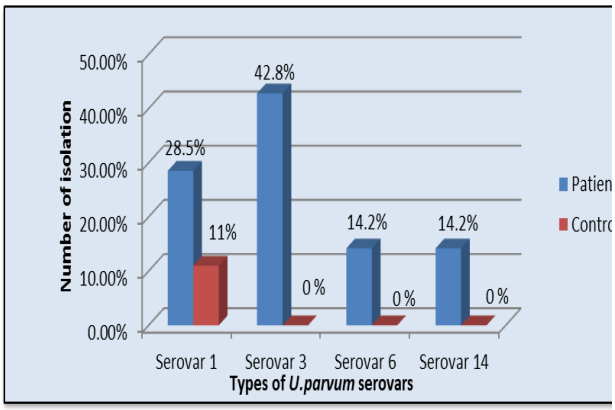

Figure -3 : Distribution of *Ureaplasma parvum* serovars among patient group and control group .

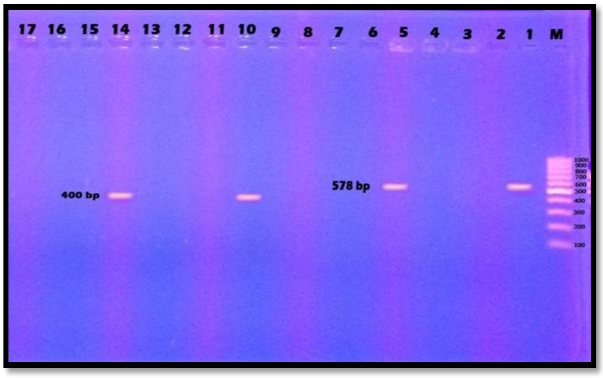

Figure -4 : Results of PCR amplification for identification of serovar 1 (578 bp) and serovar 3 (400bp).

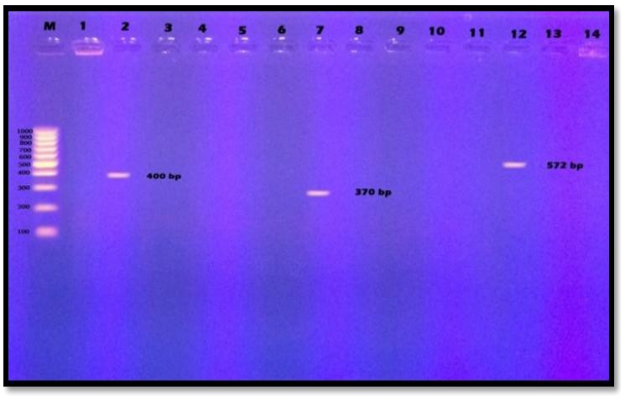

Figure -5: Results of PCR amplification for identification of serovar 3 (400 bp) , serovar 6 (370 bp) and serovar 14 (572 bp).

4.Conclusion

These results evidently indicate that demonstrated a correlation between blood group antigens and susceptibility to *Ureaplasma parvum* infections.

5.Acknowledgements

Conflicts of interest

There are no conflicts of interest .

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