Original Article

Evaluation Of Most Common Microorganisms Associated with Ectopic Pregnancy by Real Time PCR Among Iraqi Women

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Abstract

Background: ectopic pregnancy (EP) is implantation of fertilized ova outside the uterine cavity and most commonly in the tube 98% with a prevalence of 1-2% from all spontaneous pregnancies all over the world

Objective: to evaluate the most common organisms associated with tubal ectopic pregnancy isolated from female genital tract by endocervical swab using multiplex real time PCR.

Patients and Methods: a prospective cross sectional study conducted in the Department of Obstetrics and Gynaecology, Baghdad Teaching Hospital, 30 patients participate in this study, all of them diagnosed as tubal ectopic pregnancy some of them emergency cases and others are elective cases scheduled for laparoscopic surgery, endocervical swab was taken and sent for laboratory analysis by multiplex real time PCR technique using a panel of microorganisms including Niesseria gonnorhoea NG, Chlamydia trachomatis CT, Mycoplasma hominis MH, Ureaplasma Vrealyticum UU, T richomonas Vaginalis TV, Gardenerella vaginalis GV,Enterococci, and Staphylococcus aureus.

Results: The most common microorganism associated with ectopic pregnancy found by multiplex real time PCR in the iraqi patients in this study was UU 10 (33.4%), then UU+ enterococci and CT in 7 (23.3%), and one case (3.3%) with Staph Aureus and one case (3.3%) with UU+ enterococci and Staph Aureus.

Conclusion: Ureaplasma urealyticum bacteria a common organism found in the cervix of Iraqi women diagnosed with ectopic pregnancy as a uni-organism or mixed infection which calls for intensifying efforts to conduct careful screening for upper genital infections in the high risk group using endocervical swab which is easy, non-invasive, and applicable method being analysed by multiplex real time PCR for the organisms of interest which is highly sensitive procedure and the results obtained within short time which allows starting effective treatment and hence prevention of ectopic pregnancy, further researches required to determine the presence of UU and other microorganisms in the affected tubes.

Keywords: microorganisms, real time PCR, ectopic pregnancy.

INTRODUCTION

ectopic pregnancy (EP) is implantation of fertilized ova outside the uterine cavity and most commonly in the tube 98% with a prevalence of 1-2% from all spontaneous pregnancies all over the world, tubal ectopic pregnancy still considered major cause of morbidity and maternal mortality in the first 1/3 of pregnancy and responsible for about 10% from the overall maternal death in developing countries (1,2).

Since there are many predisposing factors for EP had been identified but there might be some differences between cultures, from these factors previous history of ectopic pregnancy, upper genital tract infection, previous pelvic or tubal surgery, ovarian stimulation, use on contraception devices, and smoking(3). -

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Chlamydia trachomatis is considered the most popular organism responsible for sexually transmitted infections(STI), and it is without any signs or symptoms in the majority of cases around 90%, but if the infection continuous without treatment the result will be fibrosis of the tubes, pelvic infections and ectopic gestation (4).Several microorganism have been involved in the causation of endometritis and salpingitis including chlamydia trachomatis, Neisseria gonorrhoea, mycoplasma hominis, ureaplasma urealyticum, Gardenerella vaginalis, trichomonus vaginalis(2).in addition to this group of pathogens there are a group of gram positive organisms isolated from female genital tracts like streptococcus GBS, and gram positive facultative anaerobic like spp. of enterococci, staph aureus although not a usual finding but prosperous in genital tract and involved in many infections and considered a common pathogen obtained from endocervical swabs (5). Diagnosis of STI needs a test with high detection rate, rapidity in obtaining the results, affordable, not affected by the accuracy of sample collection, and valuable in testing pathogens that are difficult to culture, from here starts the use of PCR which replaced the old methods especially multiplex polymerase chain reaction which enables diagnosis of several organisms using single sample with high detection rate and low false negatives (6).

Patients And Methods

a prospective cross sectional study conducted in the Department of Obstetrics and Gynaecology, Baghdad Teaching Hospital, 30 patients participate in this study, all of them diagnosed as tubal ectopic pregnancy some of them emergency cases and others are elective cases scheduled for laparoscopic surgery endocervical swab placed in transport media and collected in a fridge and sent for laboratory analysis by multiplex TaqMan real time PCR technique using a panel of microorganisms including Neisseria gonorrhoea, Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum, Enterococci, Staph aureus, Gargenerella vaginalis and trichomonas vaginalis.

Results Table 1 show that, thirty patients with ectopic pregnancy enrolled in the current study, half of them (50.0%) in age group between 21-30 years old, (23.3%) within the age ≤ 20 years and (26.7%) in age group (31-40) years with the mean age (27.03±6.9) years. Nil parity was found in 6 (20%) of the patients, 14 (46.7%) with 1-3 parity and 10 (33.3%) with parity >3. Only 6 (20.0%) of the patients with history of infertility. Caesarean section was the most common mode of delivery in the studied group as it found in 20 (66.7%) of the patients, then vaginal delivery in 6 (20%), and Cs+ vaginal delivery in 4 (13.3%) of the ectopic patients.

Table 1: Demographic criteria of the studied group

Variables		*No.*	*%*
Age (years)	≤ 20	7	23.3
	21-30	15	50.0
	31-40	8	26.7
Age (mean±SD)	27.03±6.9		
Parity (3.08±1.7)	nil	6	20.0
	1-3	14	46.7
	> 3	10	33.3
History of infertility	Yes	6	20.0
	No	24	80.0
Mode of delivery (n=30)	Cs	20	66.7
	Vg	6	20.0
	Cs+Vg	4	13.3

The most common microorganism found by PCR in the patients in this study was Ureaplasma 10 (33.4%), then UU+ Enterococci and CT in 7 (23.3%), and one case (3.3%) with Staph. Aureus and one case (3.3%) with UU+ Enterococci and Staph. Aureus (table 2 and figure 1).

Table 2: Distribution of the patients according to the type of microorganism infection detected by PCR

Type of microorganism infection	*No.*	*%*
Negative	11	36.7
S. Aureus	1	3.3
Ureaplasma	10	33.4
Ureaplasma+ enterococci+chlamydia	7	23.3
Ureaplasma+ enterococci and S. Aureus	1	3.3
Total	30	100%

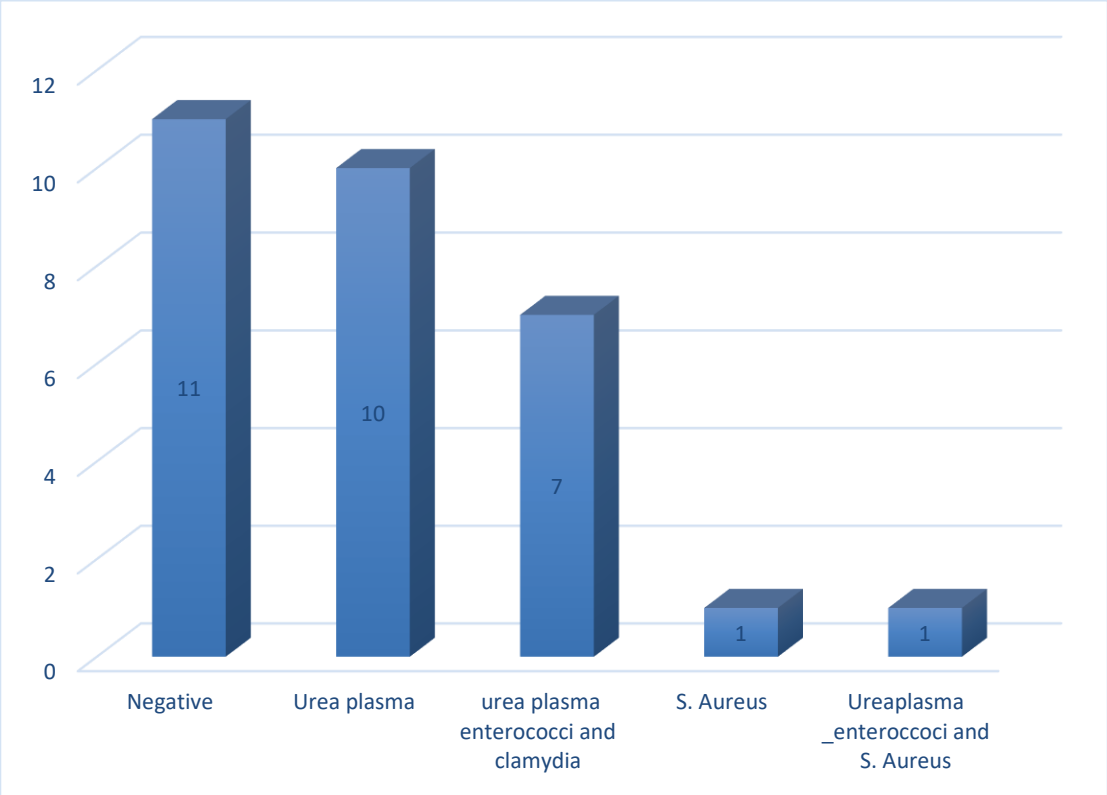

Fig.1: Distribution of the patients according to the type of microorganism infection detected by PCR

As shown in table 3, there is no significant difference between mode of delivery and type of microorganism infection (P=0.1).

Table 3: Relation between Type of delivery and microorganism in the studied group

Type of delivery	*Negative*	*Staph A.*	*Urea plasma*	*Urea plasma + Enterocoli +clamydia*	*Urea plasma + Enterocoli and S. Aureus*	*P Value*
Cs (n=20)	8	0	6	5	1	0.1 Ns
Vg (n=6)	1	0	4	1	0	
Vg+Cs (n=4)	2	1	0	1	0	

Discussion

There is a worldwide increase in the percentage of newly registered cases of sexually transmitted infections, in 2020 there were around 374 million new infections with 1 of 4 STIs: chlamydia (129 million), gonorrhoea (82 million), syphilis (7.1 million) and trichomoniasis (156 million) (7), and because of this increment and its association with PID and the effect of PID on the incidence of ectopic pregnancy we conducted this study.

In our study, we found that out of 30 cases of tubal ectopics 19 cases have been infected with one or more microorganism, 10 cases infected by ureaplasma urealyticum 33.4 %, and there are 7 cases (23.3%)have mixed infections with ureaplasma urealyticume, Enterococci and chlamydia trachomatis, and one case (3.3%) have mixed infection with ureaplasma urealyticum, Enterococci and staph aureus, and according to these results the overall ureaplasma infections reaches to 60% our PCR results includes biovar 1 and 2 and these results comparable to study conducted in china in which the UU isolated from cervical secretions of a women with ectopic pregnancy for biovar 1 (72.2%)and in biovar 2 was 11(71.4%)(8), ureaplasma urealyticum infection presented as a co-infection with CT, Enterococci spp., and staph only in 8 cases as compared to UU infection as a single organism seen in 10 cases which unlike the results obtained by a study conducted in Saudi Arabia were the prevalence of mixed infection is much more than single bacterial infection, about 20.7% had mixed infection which increases the risk of tubal conception(2). Despite UU infections seems to be the more prevalent infection in cervical secretions in Iraqi women with ectopic gestation but these results do not confirm the implication of urea plasma in tubal ectopics that's why further studies needed to confirm the presence of ureaplasm urealyticum in the upper genital tract meanwhile such association already studied (8).

One of the factors implemented in the causation of tubal ectopic is salpingitis (9), CT has been considered a main cause of salpingitis, tubal damage and ectopic pregnancy while UU and MH found as a co-infections(10), UU and MH are usually found in the vagina of healthy women as a normal commensals, meanwhile they are thought to be responsible for non-gonococcal infections such as PID(11).chlamydial infection found in our study alongside with UU and Enterococci 23.3% which is relatively comparable to same study in Saudi Arabia in which chlamydial infection found as a co –infection with mycoplasma genitalum and herpes-1/2 and the presence of more than two organisms increases the risk of tubal damage and ectopic pregnancy (2).

Staph aureus one of bacteria detected in our study by multiplex real time PCR aimed to assess the frequency of this microorganism in Iraqi women with ectopic conception and the study revealed out of thirty cases of ectopic pregnancies only two patients 6.6% showed positive results for staph infection one as a single organism and one as multiple organism although a series of epidemiological studies suggested that staphylococcus bacteria one of the organisms that is commonly isolated from genital tract either through high vaginal swabs or through cervical secretions38.7%(12). Neither Trichomonas vaginalis TV nor Gardenerella vaginalis GV detected in the cervical discharge of our patients which might be explained by the wide use of antibiotic in Iraq and the availability of antimicrobs on counter in pharmacies make these infections undetected by PCR although patient with history of antibiotic use within 2 weeks were already excluded in our study. the current study revealed the presence of enterococci species in the cervical swabs of Iraqi women with tubal pregnancy as a co-infection alongside with chlamydia and UU23.3% and with UU and staph aureus in 3.3% although enterococci, streptococcus species and E.coli isolated from vaginal secretions of women complaining from lower genital tract infection and(bacterial vaginosis) and its effect on the lactobacilli, absence of lactobacilli, and the presence of polymorphs might suggest lower genital infection(13) , this study focusing on its association with upper genital infection , salpingitis and subsequently with tubal ectopics.

Conclusion

Ureaplasma urealyticum bacteria a common organism found in the cervix of Iraqi women diagnosed with ectopic pregnancy as a uni-organism or mixed infection which calls for intensifying efforts to conduct careful screening for upper genital infections in the high risk group using endocervical swab which is easy, non-invasive, and applicable method being analysed by multiplex real time PCR for the organisms of interest which is highly sensitive procedure and the results obtained within short time which allows starting effective treatment and hence prevention of ectopic pregnancy, further researches required to determine the presence of UU and other microorganisms in the affected tubes.

Ethical approval

the study protocol was approved by the Iraqi Ethical Committee at the Departments of Obstetrics and Gynecology, College of Medicine, University of Baghdad, Iraq.

Funding

None

Conflict of interest

the author declares no conflict of interest.

Informed Consent

The patient's consent (written form) was collected from all

subjects, who enrolled in the current study.

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