*Detection of immunological markers for *Chlamydia trachomatis* and *Trichomonas vaginalis* infection in women with obstetric complications in Najaf, Iraq

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Abstract

Infection of female genital tract are associated with many obstetric and gynecological problems include abortion ,intrauterine infection , infertility ,preterm labor and postoperative infection ,in Iraq there is a little study concern with role of both Chlamydia trachomatis , *Trichomonas vaginalis* and relation with interleukin -6 in different obstetric complications. Hundred women enrolled in this study 60 with different obstetric complications include abortion ,preterm labor and intrauterine death and 40(40%) normal full term pregnancy, full history and examination was done and high vaginal swab was taken for detection of *T.vaginalis* (by wet mount preparation and antigen detection).blood was aspirated for detection of antichlamydial antibodies (IgG and IgM) and interleukin-6.The results shows that the diagnosis of T. virginals by antigen detection in vaginal swab more reliable($p<0.001$) than direct examination by wet preparation(30.0% and 6.7%) respectively. The presence of *C. trachomatis* IgG and IgM is (33.3% and 38.3% respectively) more significant ($p<0.001$) in women with obstetric complications in study group than control group (7.5 and 0.0% respectively). The highest level of *C. trachomatis* IgG and IgM was directly correlated with elevated interleukin -6 (58.3%) in women with obstetric complications.

Infection with *T.vaginalis* and *C. trachomatis* were associated with increase risk of abortion, preterm labor and intrauterine death , so screening for both infection may reduce different obstetric complications.

***The Research is apart of on Ph.D. Dissertation in the case of the third researcher**

Introduction

Trichomoniasis is a sexually transmitted infection (STI) caused by the motile parasitic protozoan *Trichomonas vaginalis*. It is one of the most common STIs, both in the United States and worldwide.(1, 2). Trichomoniasis is also associated with adverse pregnancy outcomes, infertility, postoperative infections, and cervical neoplasia.(3) *Trichomoniasis vaginalis* is one of the common parasitic infection in females. The infertile women with *T. vaginalis* with or without pathogenic microorganisms have decreased C3 and C4, increased IgA level in vaginal discharge and increased serum prolactin. (4). The annual incidence, exceeding 170 million cases per year. The disease can be difficult to diagnose due to its heterogeneous presentation and problems with diagnostic testing. All diagnostic tests are fraught with imperfections, Wet mount microscopy is the most common method of diagnosis, but requires trained observers and access to a microscope. Even in expert hands this technique is found to be only 58% sensitive (5). The other major diagnostic method, detection of the organism in culture, is considered more accurate than microscopy. However, it takes 2 to 5 days to obtain culture results, and culture also requires specialized laboratory equipment. Moreover, culture is still only 68% sensitive , and cannot be performed from urine samples (6) and the newer InPouch method may be advantageous due to simplicity in technology and cost.(7).Xenotype diagnostic kit is monoclonal antibody based detection system for *T.vaginalis* antigen that requir 10 minutes to use and can detect the presence of 10-100 organisms in 0.5 ml of vaginal fluid(8).

Chlamydia trachomatis infection, the most common reportable disease in the United States, can lead to pelvic inflammatory disease (PID), infertility, ectopic pregnancy, and chronic pelvic pain. Although *C. trachomatis* is identified among many women who receive a diagnosis of PID, the incidence and timing of PID and longterm sequelae from an untreated chlamydial infection have not been fully determined. (9). Laboratory diagnosis of *C.trachomatis* is based on culture of specimen on the cell culture media, serological diagnosis of antichlamydia antibodies, indirect fluorescent antigen test and molecular techniques such as PCR (10).*Chlamydia trachomatis* infection induces a wide array of inflammatory cytokines and chemokines, which may contribute to chlamydia-induced pathologies. However, the precise mechanisms by which *Chlamydia* induces cytokines remain unclear (11)

Material and methods

From period of February 2012 to October 2012, 100 women included in this study collected from outpatients clinic in Najaf governorate ,Iraq . the women were divided into two groups .

Group 1 : (study group) include 60 women with adverse pregnancy outcome divided into :

A. 32 women with abortion (expulsion of conception prior to 24 weeks of gestation) and include

1.incomplete abortion : when part of conception expelled and other are retained in the uterus

2.missed abortion : death of fetus before 24 weeks and remain in the uterus

3.reccurent abortion : three consecutive abortion

B. 10 women with intrauterine death (IUD):death of the fetus between 24 week until delivery

C.18 preterm delivery : delivery of the fetus before 36 completed weeks

Group2:(control group) include 40 women with full term delivery (delivery between complete 36 weeks and 40 weeks .

Complete history taken from each women include age , number of pregnancy and the outcome of each pregnancy , gestational age (by last menstrual history and ultrasound). History of pelvic inflammatory disease (PID)and whether treated or not. physical examination was done include measurement of vital sign (blood pressure ,pulse rate and temperature ,uterine size and vaginal examination for any discharge .

High vaginal swab was taken for wet preparation to detect *T.vaginalis* and *T.vaginalis* antigen by *T.vaginalis* antigen kit(8) .

Blood was aspirated form each women for detection of immunoglobulin IgG and IgM against *Chlamydia trachomatis* and interleuk-6 by ELISA assay(11) .

Result

The results revealed that most women with adverse pregnancy outcome between age group 26-35 years while most of control group between 16-25 years (table 1).most women in study group were nuliparous (n=40) while most women with full term pregnancy (control group) were multiparus. Regarding history of PID there is a significant difference ($p<0.001$) between study and control group 38 (63.3 %) of study had previous history of PID while only 8 (20.0) women in control group had history of PID .

Table 1: demographic charactertics of patients

		Adverse pregnancy outcome						Full term delivery n=40	
		Abortion n=32		P.T.L n=18		IUD n=10		NO	%
		NO	%	NO	%	NO	%		
Age (years)	16-25	15	40.9	5	27.78	1	10.0	18	45.0
	26-35	9	28.13	8	44.4	7	70.0	14	35.0
	36-45	8	25.0	6	33.3	2	20.0	8	20.0
Parity	Nulliparity	23	71.9	11	61.1	6	60.0	12	30.0
	Multiparity	9	28.13	7	38.9	4	40.0	28	70.0
PID	Positive	19	59.4	13	72	6	60.0	8	20.0
	Negative	13	40.63	5	27.8	4	40.0	32	80.0

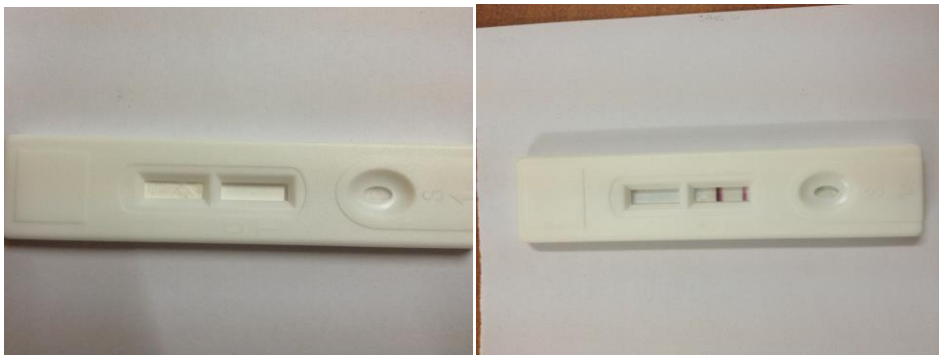
($p<0.001$)

Table 2 showed that most patients with abortion had IgG antichlamydial antibody 12 (37.5 %) more than IgM while most antibodies diagnose in women with preterm labor and IUD were IgM 10(55.6) and 6(60.0) respectively and most cases of *T.vaginalis* were diagnosed by antigen detection test rather than direct wet preparation as shown in figure 1. the level of IL-6 was high in all obstetric complications included in this study

Table 2 : relation between antichlamydial antibodies (IgG and IgM), IL-6, *T.vaginalis* Antigen and direct examination of high vaginal swab with obstetric complications among the study group (60 patients)

Obstetric complication / Diagnostic tests		Abortion (n=32)		IUD(n=10)		Preterm labor (n=18)		Total n=60	
		NO	%	NO	%	NO	%	NO	%
antichlamydial antibodies	IgG	12	37.5	2	20.0	6	33.33	20	33.3
	IgM	7	21.9	6	60.0	10	55.6	23	38.3
T.vaginalis	Wet preparation	3	9.4	0	0.0	1	5.6	4	6.7
	Ag detection	11	34.4	3	30.0	4	22.22	18	30.0
IL-6		16	50.0	7	70.0	12	66.6	35	58.3

($p<0.001$)



A: control B : positive result

Figure 1: positive result of *T.vaginalis* Antigen detected in high vaginal swab

Three types of abortion were included in this study and antichlamydial IgG Ab was higher in cases of missed abortion and recurrent abortion 6 (50.0 %) and 4(57.14%) while most patients with incomplete abortion had IgM type which indicate recent chlamydial infection. Detection of *T. vaginalis* was better by antigen detection test rather than wet preparation ($p<0.001$) and only one (7.7%) was detected of *T.vaginalis* in incomplete

abortion by wet preparation while 4(30.8%) specimens was positive by Ag detection test which is significantly different (p<0.001) regarding high level of IL-6 mostly found in case of recurrent and missed abortion 5 (71.4 %) and 7(58.3 %) respectively (p<0.001).

Table 3: relationship of *T.vaginalis* antigen , antichlamydial antibodies and IL-6 with abortion types

Abortion type / Diagnostic tests		Incomplete Abortion (n=13)		Missed abortion (n=12)		Recurrent abortion (n=7)		Total	
		NO	%	NO	%	NO	%	NO	%
antichlamydial antibodies	IgG	2	15.4	6	50.0	4	57.14	12	20.0
	IgM	4	30.8	3	25.0	0	0.0	7	11.7
T.vaginalis	Wet preparation	1	7.7	2	16.7	0	0.0	3	5.0
	Ag detection	4	30.8	4	33.33	3	42.86	11	8.3
IL-6		4	30.8	7	58.2	5	71.4	16	26.7

(p<0.001)

Also the current study showed a significant differences (P<0.001) in antichlamydial antibodies between study and control groups , detect I gM in study group was 23 (38.3 %) while in zero (0.0%) in control group and IgG in study group 20 (33.3 %) while control group only 3 (7.5 %). In regard to *T.vaginalis* 4 (6.7 %) was diagnosed by wet preparation in study group and only one (2.5 %) diagnosed in cotrol group and 18 (30.0 %) detected by antigen detection in HVS in study group and only 3 (7.5 %) in control group which significantly deferent (P<0.001). the level of Il-6 was significantly high in study group 35(58.3 %) while only 2 (5.0 %) is high in control group which is shown in table 4.

Table 4 : difference between study and control groups according to immunological parameters and direct HVS examination for *T.vaginalis*

Obstetric complication / Diagnostic tests		Study group (n=60)		IUD(n=40)	
		NO	%	NO	%
antichlamydial antibodies	IgG	20	33.3	3	7.5
	IgM	23	38.3	0	0.0
T.vaginalis	Wet preparation	4	6.7	1	2.5
	Ag detection	18	30.0	3	7.5
IL-6		35	58.3	2	5.0

(P<0.001)

Discussion

Pelvic inflammatory disease (PID) is one of the most serious infections facing women today. It is a common problem encountered in gynecologic infertility, family planning, postnatal, legal abortions, and sterilization clinic in India and abroad.(12).The identification of the risk factors associated with PID is crucial to efforts for the prevention of these consequences.(13).Many reportes mention that abortion could have resulted from a sexually transmitted infection/disease that was present at the time of the abortion. It is indeed likely that following an abortion the main risk to fertility is the development of Pelvic Inflammatory Disease (PID), which is an inflammation of uterus, fallopian tubes and ovaries. Any use of instruments on the cervix, such as during a D&C, can lead to a greater spread of these organisms and, therefore, the risk of PID(14), this agreement with our results that 59.4% cases of abortion have positive history of PID.The most common means of trichomoniasis diagnosis is visualization of the motile trichomonads in a saline preparation of the vaginal fluid. Although quick and inexpensive, the test has limited sensitivity, ranging from 60 to 70% (15).

More recently, a point-of-care antigen detection test for the diagnosis of trichomoniasis in women has been licensed (Genzyme Corp. Cambridge, Mass.). Rapid test performance did not vary with vaginal symptoms or with the presence of other vaginal or cervical syndromes or infections. The rapid assay was more sensitive than wet-preparation microscopy (78.5 and 72.4%, respectively; P = 0.04) but was less specific (98.6 and 100.00%, respectively; P = 0.001) (16). These results similar to the current study which show only 6.7% of cases of trichomoniasis diagnosed by wet preparation while 30% (p=0.001) positive in rapid antigen detection.In other study also shows the XenoStrip-Tv (Xenotope Diagnostics, Inc., San Antonio, Tex.) was evaluated on vaginal swab specimens from 936 women attending sexually transmitted disease clinics in Seattle, Wash**.** and Birmingham, Ala *T. vaginalis* prevalence by culture (InPouch; Biomed) was 8.7% in Seattle and 21.0% in Birmingham. Compared to culture, the XenoStrip assay in Seattle was 76.7% sensitive and 99.8% specific, and in Birmingham it was 79.4% sensitive and 97.1% specific. The sensitivity did vary by day of culture-positive result, with a 71% decline in XenoStrip sensitivity for every additional day delay until *T. vaginalis* was first detected in cultures . The rapid assay was more sensitive than wet preparation microscopy (78.5% versus 72.4% (P = 0.04)but was less specific (98.6% versus 100% (P = 0.001). The XenoStrip rapid assay is well suited for use in settings with a moderately high prevalence of *T. vaginalis* infection, particularly when microscopy is not practical(17).Genital chlamydial infection is a sexually transmitted disease (STD) of public health importance,although asymptomatic infections are quite common, occurring in up to 70% of infected women and 50% of infected men (18,19).

The most useful demographic or behavioural risk factor for genital chlamydia is age. Adolescents, and young men and women are consistently at higher risk of being infected with chlamydia than older subjects(20).All sexually active women 24 years of age or younger, including adolescents, are at increased risk for chlamydial infection. and this similar to our finding that most women with adverse pregnancy outcome and had trichomoniasis chlamydial infection are young.The current study shows 37.5% of abortion cases had positive antichlamydial IgG and 21.9 % had positive antichlamydial IgM , this goes with a study which declare that about 20 to 27% of patients seeking abortion have a chlamydia infection. Approximately 5% of patients who are not infected by chlamydia develop PID within 4 weeks after a first trimester abortion(21).

Studies demonstrate the high prevalence of chlamydial infections in the general U.S. population. Based on estimates from national surveys conducted from 1999–2008, chlamydia prevalence is 6.8% among sexually active females aged 14–19 years.(22). While the current study shows 33.3% chlamydial IgG and 38.3 % chlamydial IgM respectively in study group while 7.5% and 0.0% chlamydial IgG and chlamydial IgM in control group. In patients with 1 miscarriage chlamydia infection by means of PCR was detected in 11.8% of women (p=0.029), in patients with > or =2 miscarriages in 9.1% (p=0.198) and in the comparative group in 2.2%. Specific anti-chlamydial antibodies IgA class were detected in: 7.9 (p=0.082) in group 1, 4.5% (p=0.236) in group 2 and in 0% in group 0, and IgG class in 21.1% (p=0.024), 36.4% (p=0.000) and in 4.4%, respectively(23). While current study found 57.14% of women with recurrent abortion have positive chlamydial IgG and the 7.5 % of control group had positive chlamydial IgM.We found that 33.3% and 55.6 for chlamydial IgG and chlamydial IgM positive respectively in women with preterm labor, these finding were similar to study concern with,*Chlamydia trachomatis* infection contributes significantly to early premature delivery and should be considered a public health problem, especially in young women and others at increased risk of *C. trachomatis* infection(24).

Also the current study shows high level of IL-6 in women with obstetric complication cased by *Chlamydia trachomatis* infection, this in concordance with a study shows that IL-1 Beta, IL-4, IL-5, IL-6 and IL-10 levels were found to be higher in *Chlamydia trachomatis* positive women with fertility disorders compared to *Chlamydia trachomatis* positive fertile women and controls ($P < 0.05$) (25).

Also other study done in Germany, suggested that interleukin (IL)-6 and IL-6 receptors founded in histopathology specimens from infants who died preterm show an association between intraventricular haemorrhage (IVH), periventricular leucomalacia and infection. Also demonstrated that extremely immature preterm infants have a high expression of IL-6 and IL-6 receptors in the ganglionic eminence of the brain resulting in high protease activity and leading to vascular fragility and intracranial haemorrhage. (26).

We conclude that infection with *T.vaginalis* and *C. trachomatis* are associated with increased risk of abortion, preterm labor and intrauterine

death , so screening for both infections may reduce different obstetric complications in Iraq.

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*التحري عن الدلائل المناعية لاصابات بكتريا *Chlamydia trachomatis* والمشعرات المهبلية *Trichomonas vaginalis* لدى النساء اللواتي لديهن مضاعفات الحمل في محافظة النجف ، العراق

تاريخ الاستلام : ٢٠١٢\٩\١٤ تاريخ القبول : ٢٠١٢\١١\٢٧

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الخلاصة :

ان التهاب القناة التناسلية الانثوية مرتبط بعدد من المشاكل النسائية تتضمن الاسقاطات والتهاب الرحم والقعم والولادة المبكرة والتهاب بعد العمليات. توجد دراسات محدودة في العراق مهتمة بدور التهاب الكلاميدية والمشعرات المهبلية ودور الانترليوكين ٦ بمختلف مضاعفات الحمل . شملت الدراسة ١٠٠ امراة تضمنت ٦٠ امراءة تعاني من احدى مضاعفات الحمل ومنها الاسقاطات والولادة المبكرة وموت الجنين في الرحم بعد الشهر الخامس و٤٠ امراءة (مجموعة سيطرة) مع حمل طبيعي في الشهر التاسع دون مشاكل .تم اخذ التاريخ المرضي لكل حالة واجري لكل امراءة فحص سريري كما اخذت عينة من اعلى المهبل للتحري عن المشعرات المهبلية باستعمال الفحص المباشر للعينة الرطبة وباستخدام التحري عن المستضد للمشعرات المهبلية ، ثم تم سحب الدم من كل امراءة للتحري عن الاجسام المضادة للكلاميدية لكلا النوعين الامينو كلوبيولين IgG وIgM وكذلك للتحري عن الانترليوكين ٦ . اظهرت النتائج ان تشخيص المشعرات المهبلية باستخدام تقنية التحري عن المستضد في مسحات المهبل معتمدة اكثر($p<0.001$) من الفحص المباشر للعينة الرطبة (٣٠.٠% و٦.٧%) على التوالي . ان وجود الاجسام المضادة الكلاميدية لكلا النوعين الامينو كلوبيولين IgG وIgM في النساء المصابات بمضاعفات الحمل (٣٣.٣ % و ٣٨.٣%) اكثر ارتباطا ($p<0.001$) من النساء مع الحمل الطبيعي (٧.٥% و ٠.٠%) . ان المستوى العالي للاجسام المضادة للكلاميديا لكلا الصنفين يرتبط بصورة مباشرة مع ارتفاع نسبة الانترليوكين ٦ في النساء المصابات بمضاعفات الحمل(٥٨.٣). ان الاصابة بالالتهاب الكلاميديا والمشعرات المهبلية مرتبطى بزيادة نبة الاسقاطات والولادات المبكرة وموت الجنين في الرحم لذلك فان استخدام المسح للكشف عن هذه الالتهابات قد يقلل من المشاكل التي تحدث في الحمل .

***البحث مستل من أطروحة دكتوراه للباحث الثالث**