

## Study the role of Chlamydia trachomatis in ectopic pregnancy in Iraqi women

دراسة دور بكتريا Chlamydia trachomatis في الحمل خارج الرحم في النساء العراقيات

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### Abstract

The aim of this study was to investigate the role of Chlamydia trachomatis as a risk factor in ectopic pregnancy in Iraqi women. The study involves two groups, the first group included 24 women diagnosed as ectopic pregnancy, while the second group included 24 women as control group diagnosed as normal pregnancy. Both groups were admitted to Al-Elweya Teaching Hospital, Baghdad from November 2009 to July 2010. Serum antichlamydial antibodies were investigated in both studied groups using enzyme linked immunosorbent assay (ELISA). Results revealed a significant ( $p < 0.05$ ) percentage of IgG antichlamydial antibodies in women detected as ectopic pregnant, as compared with control normal pregnant (45 vs. 8.3%). On the other hand, ectopic pregnant women exhibited low ( $p < 0.05$ ) IgM antichlamydial antibodies 4% as compared with control group. In conclusion all women with seropositive to Chlamydia trachomatis antibodies had pelvic inflammatory disease (PID).

### المستخلص

هدفت الدراسة الى التحري عن دور بكتريا Chlamydia trachomatis لحالات الحمل خارج الرحم في النساء العراقيات. تضمنت الدراسة مجموعتان، المجموعة الأولى شملت 24 امرأة مشخصة كحمل خارج الرحم اما المجموعة الثانية فشملت 24 امرأة مشخصة كحمل طبيعي واعتبرت مجموعة السيطره. توافدت المجموعتان الى مستشفى العلوية التعليمي في بغداد في الفتره تشرين الثاني 2009 ولغاية تموز 2010. تم التحري عن الأجسام المضاده نوع (IgG, IgM) لبكتريا Chlamydia trachomatis في مصول الفنتين قيد الدراسة باستخدام تقنية الأليزا. أظهرت النتائج ارتفاعا معنويا ( $p < 0.05$ ) في النسبه المنويه للأجسام المضاده نوع G لبكتريا Chlamydia trachomatis في مصول النساء المشخصة كحمل خارج الرحم بالمقارنة مع مجموعة السيطرة (45 vs. 8.3%). من ناحيه اخرى اظهرت حالة الحمل خارج الرحم انخفاض ( $p < 0.05$ ) في مستوى الأجسام المضاده نوع M لبكتريا Chlamydia trachomatis 4% مقارنة مع مجموعة السيطرة. يمكن الاستنتاج ان جميع النساء اللواتي اظهرن نتائج مصليه موجبه للأصابه يعانين من التهاب منطقة الحوض (PID).

### Introduction

Ectopic pregnancy (EP) is defined as a pregnancy in which the implantation of the embryo occurs in the early first weeks outside the uterine cavity, most frequently in one of the two fallopian tubes or, more rarely, in the abdominal cavity [1] while in normal pregnant cases the sperm fertilizes the ovum in fallopian tube, the zygote passes to be implanted in the uterus to grow [1, 2].

Over the last 20 years the incidence of EP has shown a significant increase in many countries. In parallel, an increase in the incidence of sexually transmitted disease (STD) has been shown, [3]. An early ectopic pregnancy often feels like a normal

pregnancy, but when the EP progresses other symptoms develop including (Abdominal or pelvic pain, vaginal bleeding, Dizziness and fainting and signs of shock) [2].

The major causes of EP are pelvic inflammatory disease (PID) which is caused by bacteria such as Chlamydia trachomatis and gonorrhoea. Other causes of EP include the use of contraceptive, surgery and smoking [14].

Many recent reports indicate that Chlamydia trachomatis is the major causative agent in development of PID and EP more importantly than gonococcal infection [4,5]. Chlamydia trachomatis is an obligate intracellular gram negative bacterium and it is the most common bacterial sexually transmitted disease (STD) worldwide, especially among young adults. Infection with this agent can be asymptomatic in up to 80% of women which can make diagnosis and detection difficult [4,6]. Left undetected and untreated Chlamydia can ascend the upper genital tract where they colonize the endometrial mucosa and the fallopian tubes, leading to (PID). Chlamydial PID can cause tubal occlusion or partial occlusion with an increased risk for ectopic pregnancy because these regions become blocked and keep the embryo from reaching uterus [7,8].

The link between genital Chlamydia trachomatis infection and EP has mainly been established through seroepidemiologic studies in which selected groups of patients with ectopic pregnancy have been compared with normal pregnant women due to infection with Chlamydia trachomatis that will result in the formation of G antibody detectable in serum in chronically infected women [9,10,11].

Many report indicated that the serological test of Chlamydia trachomatis antibodies are not cost effective and beneficial, while isolation of this agent by cell culture has not been applied to date [11,12]. So, the goal of this study was to investigate a role of Chlamydia trachomatis as a risk factor in EP.

### **Materials and Methods**

Fourty eight pregnant women attending to Al-Elweya Teaching Hospital in Baghdad were selected. Pregnant women were divided in two groups depending on: Ultra sonograph "Ultra sound" and determination of hCG hormone level. When hCG level > 1500 ml U/ml, that's mean ectopic pregnancy, while hCG level < 1500 ml U/ml mean normal pregnancy [7].

The first group included 24 cases of ectopic pregnancy women (implantation of embryo occurs outside the uterine cavity, hCG > 1500 ml U/ml).

The second group included 24 cases of normal pregnant women (implantation of embryo occurs inside the uterine cavity, hCG <1500 ml U/ml).

Information about age, city, numbers of children, previous EP, frequent of pregnancy, has PID, use of contraceptive and smoking was collected.

The clinical diagnosis for PID was based on the following criteria:

Acute pelvic pain, especially in the postmenstrual period, fever, abnormal cervical discharge, uterine/ adnexial tender-ness, and cervical motion tenderness [7,8].

Blood samples were taken and sera were used for:

1. Determination of IgG, antibodies against Chlamydia trachomatis was done by using ELISA technique (DRG, Germany). All assays and calculations were performed according to the manufacturer's instructions.
2. Determination of IgM antibody against Chlamydia trachomatis by ELISA kit (DRG, Germany).

The principle of the test and assay procedure similar to the determination of IgG antibody but there is one difference the patient samples are diluted with sample diluents and additionally incubated with IgG-RF-Sorbent, containing hyper-immune anti-human IgG-Class antibody to eliminate competitive inhibition from specific IgG and to remove rheumatoid factors.

### Principle of the test

The DRG Chlamydia trachomatis IgG ELISA kit is a solid phase enzyme-linked immune sorbent assay (ELISA). Micro titer wells as a solid phase are coated with recombinant Chlamydia trachomatis antigen. Diluted patient specimens and ready-for-use controls are pipetted into these wells. During incubation Chlamydia trachomatis specific antibodies of positive specimens and controls are bound to the immobilized antigens. after a washing step to remove unbound sample and control material horse radish peroxidase conjugated anti-human IgG antibodies are dispensed into the wells. During a second incubation, this anti-IgG conjugate binds specifically to IgG antibodies resulting in the formation of enzyme-linked immune complexes.

After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive result) are detected by incubation with TMB substrate and development of a blue color the blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid.the intensity of this color is directly proportional to the amount of Chlamydia trachomatis specific IgG antibody in the patient specimen, absorbance at 450 nm is read using an ELISA micro titer plate reader.

The results calculated according to this formula.

$$\frac{\text{Patient (mean) absorbance value} \times 10}{\text{Co}} = [\text{DRG units} = \text{DU}]$$

Co: Mean absorbance value of cut-off control.

- 2- Determination of IgM antibody against Chlamydia trachomatis by ELISA kit (DRG, Germany).

The principle of the test and assay procedure similar to the determination of IgG antibody but there is one difference the patient samples are diluted with sample diluents and additionally incubated with IgG-RF-Sorbent, containing hyper-immune anti-human IgG-Class antibody to eliminate competitive inhibition from specific IgG and to remove rheumatoid factors.

### Result and Discussion

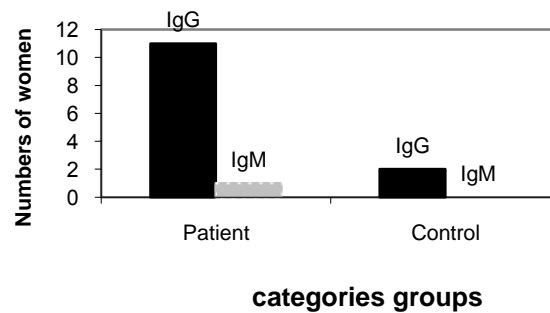
The prevalence of Chlamydia IgG antibodies was significantly higher in group I (11/24, 45%) as compared with group II (2/24, 8.3%) ( $p < 0.05$ ) ( $X^2 = 8.545$ ).

The seropositive association between Chlamydia antibodies and EP has also been reported [4, 3, 12, 14] those found that 50% of women with EP were seropositive to

Chlamydia trachomatis. Some studies demonstrated that women who had Chlamydia trachomatis were 7-10 fold increased risk for EP. This is due to Chlamydia infections can ascend to the upper genital tract, where they colonize the endometrial mucosa and the fallopian tubes and cause tubal occlusion with increased risk for EP [7].

Based on the correlation between Chlamydia IgG antibody and EP, some authors suggest that testing for these antibodies should be a part of the basic routine investigation in infertility clinics [15].

In Only 1/24 (4%) women serum of group I IgM antibody was detected. The decrease in IgM, antibodies percentage in Chlamydial infection is due to conventional IgM to IgG switch. Figure (1):



**Fig (1): Distribution of anti Chlamydia antibodies with categories groups:**

**IgG: Chi square = 8.545; D.F. = 1; P<0.05**

**IgM: Chi square = 1.021; D.F. = 1; P> 0.05**

These results are in agreement with other studies that reported the prevalence of IgM in women with EP was 3% [16]. Our results were disagree with other studies those found high prevalence of IgM antibody to C.T. [17].

Sixteen women in group I (66%) reported PID history compared with 4 (15%) in group II. These results were statistically significant (P<0.05). That mean all women with seropositive for Chlamydia IgG had PID history.

Our results are in agreement with other studies [4,7], which found women with untreated Chlamydia go on to develop pelvic inflammatory disease, which can destroy the delicate hair-like cells lining the fallopian tubes. Severe infections can scar the tubes causing them to become blocked. Women with PID have five times greater risk for EP [18].

In this study higher proportion (83%) of patients than controls (20%) consistal of smokers at the time of conception (P<0.05) ( $X^2 = 18.783$ ).

Result from previous studies lend support that smoking is a risk factor for the occurrence of tubal pregnancy [19]. This may be due to the nicotine in cigarettes. Nicotine can cause temporary blockage of the tube so the embryo cant pass through. [20].

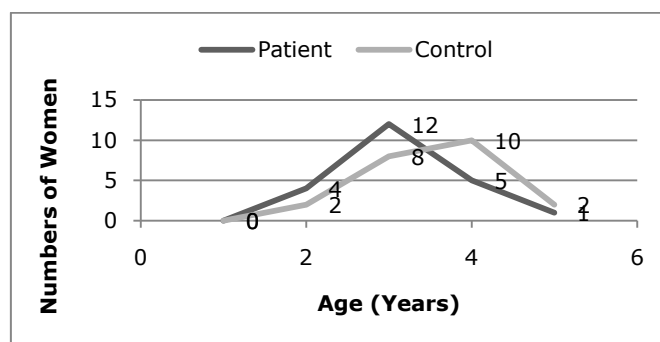
**Table(1): Characteristics of women in the two study groups:**

| Characteristics                      | Group I Ectopic (N = 24) | Group II Normal (N = 24) |
|--------------------------------------|--------------------------|--------------------------|
|                                      | N (%)                    | N (%)                    |
| Antichlamydial IgG<br>POSTIVE        | 11 (45%)                 | 2 (8.3%)                 |
| Anti chlamydial IgM<br>POSTIVE       | 1 (4%)                   | (0%) 0                   |
| PID history                          | 16 (66%)                 | 4 (15%)                  |
| Previous (E.P.) Ectopic<br>pregnancy | 14 (58%)                 | 2 (8.3%)                 |
| Previous (N.P.) Normal<br>pregnancy  | 10 (41%)                 | 18 (75%)                 |
| Use of Contraceptive                 | 14 (58%)                 | 4 (15%)                  |
| Smoking                              | 20 (83%)                 | 5 (20%)                  |
| History of abortion                  | 5 (20%)                  | 4 (16%)                  |

Fourteen (58%) of the patient with ectopic pregnancy use contraceptive when compared with normal pregnant women (4, 15%) ( $P < 0.05$ ) ( $\chi^2 = 8.889$ ). This result has been reported by the other author [9] because the use of contraceptives are most likely reflected in increased risk for pelvic infection, scarring of the fallopian tubes, tubal occlusion and Ectopic pregnancy [15]. Other studies [3] did not found any correlation between the use of contraceptive and EP.

Five of women in group I (20%) and 4 (16%) of group II had a history of abortion, none of these differences were statically significant.

Our result was in disagreement with other results those found high proportion of patients and controls had a history of abortion [20]. Table (1) shows that women with an ectopic pregnancy were more likely to have had previous tubal pregnancy (14/24) (58%) than women with intra-uterine pregnancy (2/24) (8.3%). These results are in agreement with other studies which found that 60% of women with EP had previous EP [8, 15]. The mean age for group I was 30 years, while for group II was 34 years. These result are in line with other studies [3,7] those found that the pregnancy are common among these ages which considered sexually active ages of women. Figure(2).

**Fig (2): Disease frequency according to age groups of studied women**

The present data reinforce the need for public health policies in our countries, which promise triage and eventual treatment for young women with C.T. genital infections, thus avoiding the serious sequelae to women's reproductive health and a reduction in the financial burden hospital commitment.

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