KJMS / OPEN ACCESS Print ISSN: 2790-0207 Online ISSN: 2790-0215

Original article

Anti-Chlamydial Antibodies In Women with Ectopic Pregnancy

DR. Sahar Kamal Jalal[1,*], Professor Ayla Khedher Ghalib[2]

[1] MB CH B, Iraqi Board for Medical Specialization, Iraq

[2] Department of Obstetrics and Gynecology, College of Medicine, University of Kirkuk, Kirkuk, Iraq

*Corresponding author Email address: saharkamaljalal19@gmail.com

DOI: 10.32894/kjms.2022.174201

Abstract:

- ***Background*:** *Chlamydia trachomatis* is the most prevalent bacterial sexually transmitted infection globally and is associated with serious reproductive complications, including ectopic pregnancy. The presence of anti-chlamydial immunoglobulin G (IgG) antibodies has been linked to a higher risk of ectopic pregnancy. This study aimed to compare the frequency of *Chlamydia trachomatis* infection in women with ectopic pregnancy versus those with normal intrauterine pregnancies.
- ***Methods:*** A case-control study was conducted over 10 months (February–December 2019) at the Department of Obstetrics and Gynecology, Azadi Teaching Hospital, Kirkuk, Iraq. A total of 86 pregnant women were enrolled: 43 with confirmed ectopic pregnancy (case group) and 43 with early normal intrauterine pregnancies (control group). Women with a history of ectopic pregnancy, infertility, tubal surgery, smoking, or intrauterine device use were excluded. Serum anti-chlamydial IgG titers were measured in all participants.
- ***Result:*** There were no statistically significant differences between the groups regarding age, gestational age, body mass index, or parity. Anti-chlamydial IgG was positive in 39.5% of women with ectopic pregnancy compared to 16.3% in the control group. The mean antibody titer was significantly higher in the ectopic pregnancy group. A titer above 9.98 NTU may predict the risk of ectopic pregnancy. No significant correlations were found between antibody titer and demographic or clinical parameters.
- ***Conclusions:*** A higher frequency and titer of anti-chlamydial antibodies were observed in women with ectopic pregnancy. These findings suggest a potential role of chlamydial infection in the pathogenesis of ectopic pregnancy.
- **Keywords:** Ectopic pregnancy, Chlamydia trachomatis, Anti-chlamydial IgG antibodies

INTRODUCTION

An ectopic or extrauterine pregnancy is one in which the blastocyst implants anywhere other than the endometrial lining of the uterine cavity. Nearly 95 percent of ectopic pregnancies (EP) implant in the fallopian tube. Bilateral EPs are rare, with an estimated prevalence of one in every 200,000 pregnancies (1). In the United Kingdom, the estimated maternal mortality associated with EPs is 0.2 per 1000 cases (2). Over 95% of EPs are tubal in origin, with 80% located in the ampullary portion of the fallopian tube (3).

Only about 50% of women diagnosed with an EP have identifiable risk factors (4). These include a previous history of EP, pelvic surgery, pelvic inflammatory disease (PID), *Chlamydia trachomatis* infection, smoking, intrauterine contraceptive device use, in-utero exposure to diethylstilbestrol, assisted reproductive technology, and maternal age. *Chlamydia trachomatis* infection is the most common sexually transmitted bacterial infection worldwide (5). It often causes milder symptoms compared to other sexually transmitted diseases (6), allowing it to go unnoticed until secondary or tertiary complications develop.

Serious consequences such as acute salpingitis and PID are frequently associated with repeated or persistent infections (7). Despite widespread screening and treatment efforts, the *Chlamydia* epidemic persists, with a continual increase in reported cases. Urogenital chlamydial infection can result in adverse outcomes in women, including PID, which may lead to tubal factor infertility, ectopic pregnancy, and chronic pelvic pain. Notably, chlamydial infections do not confer lasting immunity (8).

Infections caused by *C. trachomatis* induce the formation of serum-detectable antibodies. While "chlamydia antibodies" broadly refer to antibodies against the *Chlamydia* genus, they specifically target *C. trachomatis* in human infections (9). In cases of lower genital tract infections, the detection of these antibodies in a single serum specimen is of limited value, as they may be present even in women without active infection. However, there is considerable evidence linking the presence of these antibodies—particularly at high titers—with upper genital tract infections (10).

Acute inflammation is implicated in the tubal damage that predisposes to EP. Conditions such as chronic salpingitis and salpingitis isthmica nodosa also contribute. Recurrent chlamydial infections lead to intraluminal inflammation, fibrin deposition, and tubal scarring. Persistent chlamydial antigens may provoke a delayed hypersensitivity response, perpetuating damage

despite negative cultures. Inflammation within the fallopian tube may disrupt embryo transport and produce premature implantation signals (11).

As the fallopian tube lacks a submucosal layer, a fertilized ovum can easily implant within the muscularis layer. Rapid proliferation of trophoblasts can erode the muscularis, allowing maternal blood to fill surrounding spaces, leading to rupture (12).

Several studies have shown a higher proportion of women with EP have serologic evidence of past *C. trachomatis* infection compared to women with intrauterine pregnancies. Among those with EP, seropositive patients had a higher prevalence of pelvic adhesions compared to seronegative patients. Furthermore, increasing antibody titers were significantly associated with both the presence and severity of tubal damage (13).

PATIENT and METHOD

This case-control study was conducted in the Department of Obstetrics and Gynecology at Azadi Teaching Hospital, Kirkuk, over a 10-month period from February 1st to December 1st, 2019. Verbal consent was obtained from all participants prior to enrollment.

A total of 90 pregnant women were initially recruited from both the outpatient clinic and emergency/labor wards. Four participants were excluded due to invalid or missing anti-chlamydial antibody titer results, leaving 86 women eligible for final analysis. The participants were categorized into two groups:

- **Case group:** 43 women diagnosed with tubal ectopic pregnancy.
- **Control group:** 43 women with early normal intrauterine pregnancies (first trimester), matched for age and gestational age with the case group.

The diagnosis of ectopic pregnancy was based on the following criteria:

- Serum beta-human chorionic gonadotropin (β-hCG) level > 1500 IU/mL.

- Transvaginal ultrasound findings showing an empty uterine cavity and cervical canal, but with a gestational sac located in the adnexa or fallopian tube.
- All scans were performed by an obstetrician and reviewed by a radiologist. In some acute presentations, diagnosis was confirmed intraoperatively and supported by histopathological findings.

Control participants were selected based on clinical signs of early pregnancy (e.g., menstrual delay), positive pregnancy test, and ultrasound confirmation of a normal intrauterine gestational sac.

Exclusion criteria included:

- Use of intrauterine contraceptive devices (IUCD) at the time of conception
- Previous ectopic pregnancy
- History of infertility or tubal surgery
- History of in vitro fertilization (IVF)
- History of smoking
- Other types of ectopic or heterotopic pregnancies

All participants completed a structured questionnaire to obtain demographic and clinical data. General examination including measurement of body mass index (BMI) was performed.

Laboratory and imaging investigations included:

- Full blood count
- Serum pregnancy test
- β-hCG titer

- Serum anti-chlamydial immunoglobulin G (IgG) antibody levels
- Transvaginal ultrasound to assess gestational sac location, size, and presence of free fluid

A positive anti-chlamydial IgG result was defined as a titer > 11 NTU.

RESULTS

A total of 86 pregnant women were included in the study. They were equally divided into two groups: the case group consisted of 43 women diagnosed with tubal ectopic pregnancy, while the control group included 43 women with early normal intrauterine pregnancies.

As shown in Table 1, there were no statistically significant differences between the two groups in terms of age, body mass index (BMI), gestational age (GA), or parity ($P \geq 0.05$), indicating adequate matching between groups for these variables.

Table 1: Comparison between study groups by certain characteristics

Variable	Case Mean ± SD	Control Mean ± SD	P – Value
Age (Year)	28.39 ± 5.8	27.72 ± 6.4	0.609
GA (Week)	6.46 ± 0.66	6.76 ± 1.3	0.167
BMI (kg/m²)	25.3 ± 2.6	25.67 ± 3.0	0.545
Parity	1.97 ± 1.5	1.69 ± 1.0	0.313
Anti-chlamydial antibody titer (NTU)	11.72 ± 1.81	9.36 ± 2.05	**0.001**

The comparison of anti-chlamydial antibody results between the groups is presented in Figure 1 and Table 1. Among women with ectopic pregnancy, 17 (39.5%) tested positive for anti-chlamydial IgG antibodies, whereas only 7 (16.3%) of the control group tested positive.

Furthermore, the mean anti-chlamydial antibody titer was significantly higher in the ectopic pregnancy group compared to the control group (11.72 NTU vs. 9.36 NTU, P = 0.001).

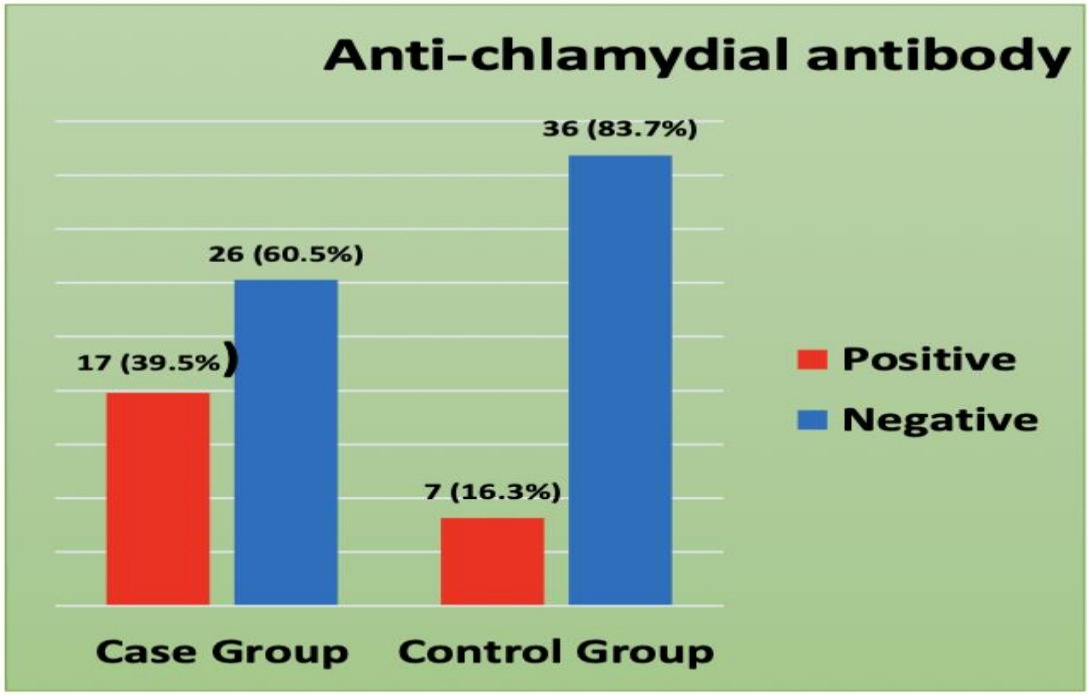

Figure 1 . Anti-chlamydial Antibody Results in Study Groups

A receiver operating characteristic (ROC) curve analysis was performed to evaluate the predictive value of anti-chlamydial antibody titer for ectopic pregnancy. As shown in Figure 2 and Table 2, a cutoff value of 9.98 NTU was identified. An antibody titer above this threshold was significantly associated with increased risk of ectopic pregnancy, with an area under the curve (AUC) of 84.6%.

Table 2: Diagnostic Accuracy for Test of Ectopic Pregnancy

	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy
Anti-chlamydial antibody titer (NTU)	9.98	88.4%	81.4%	82.6%	87.5%	84.9%

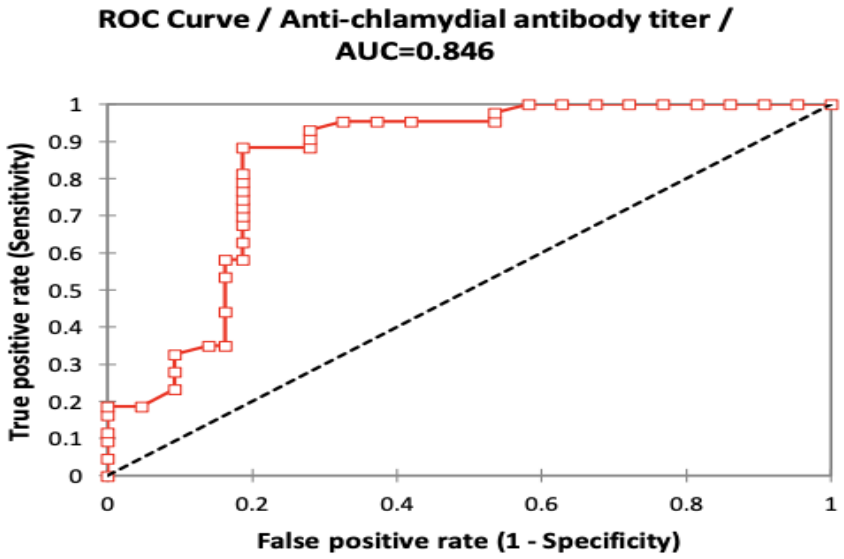

Figure 2 : ROC curve for anti-chlamydial antibody titer as a predictor for the risk of ectopic pregnancy

These findings suggest that elevated anti-chlamydial antibody titers may serve as a useful predictive marker for ectopic pregnancy.

No statistically significant correlations ($P \geq 0.05$) were observed between anti-chlamydial antibody titers and participants' age, BMI, or gestational age (Table 3).

Table 3: Correlation between anti-chlamydial antibody titer and certain characteristics

Variable	r	P – Value
Age (year)	0.035	0.75
BMI (kg/m²)	0.009	0.938
GA (Week)	- 0.059	0.592

DISCUSSION

Ectopic pregnancy (EP) remains a significant public health concern, particularly in many developing nations where it contributes considerably to pregnancy-related morbidity and mortality (14). This is supported by its increasing incidence worldwide (15). The primary risk factors for tubal EP include tubal damage from previous surgery or infection, smoking, and assisted reproductive technologies. Another proposed mechanism involves alterations in the transport mechanism or environment within the fallopian tube, leading to fetal retention (16).

Numerous studies have investigated the antibody responses triggered by *Chlamydia trachomatis* infection, consistently showing a strong association between the presence of serum antibodies and the risk of EP. The risk appears to be especially elevated in women with repeated *Chlamydia* exposures. These findings emphasize that *C. trachomatis* infection is a major contributor to fallopian tube damage, which predisposes individuals to EP (17).

Although the levels of *C. trachomatis* IgG antibodies decline over time following infection they may persist at detectable levels for several years—even after successful antibiotic

treatment. This persistence is more pronounced in women with repeated infections. Moreover, a correlation has been demonstrated between antibody titers and the severity of inflammation and tubal pathology (18,19).

In this study, 86 women were enrolled—43 in the case group (tubal EP) and 43 in the control group (first-trimester normal intrauterine pregnancy). Anti-chlamydial antibody positivity was observed in 39.5% of the EP group, compared to 16.3% in controls. The mean antibody titer was significantly higher among EP patients (11.72 vs. 9.36 NTU, P=0.001). However, there were no statistically significant correlations between antibody titer and parity, age, BMI, or gestational age ($P \geq 0.05$).

These findings are consistent with those of Rantsi et al. (20), who reported a higher seropositivity for *C. trachomatis* IgG in EP cases (29.3%) compared to controls (15.0%, $P < 0.001$). Similarly, Agholor et al. (21) found a significant difference in *Chlamydia* antibody positivity between EP patients (48.0%) and controls (16.3%, $P < 0.001$), though they also noted that the presence of antibodies was not significantly associated with parity ($P > 0.05$).

In contrast, Bokhari et al. (22) observed no statistically significant difference between cases and controls in anti-chlamydial IgG prevalence (25% vs. 11.3%, $P = 0.097$), though a higher proportion was still seen among cases. They also reported no significant association between anti-*Chlamydia* IgG and parity or contraceptive method.

Discrepancies among studies may be due to differences in sample size, disease duration and virulence, antibody titers, and histories of pelvic inflammatory disease (PID) or previous surgeries. Notably, antibody levels can wane over time, making detection difficult in some cases (23).

In this study, ROC curve analysis identified a titer cut-off of >9.98 NTU as predictive for EP, with a sensitivity of 88.4%, specificity of 81.4%, and overall accuracy of 84.9%. The pathogenesis of *C. trachomatis*–related reproductive issues is believed to involve

inflammation-induced mucosal damage and scarring (24). Both innate and adaptive immune responses contribute to disease severity, with genetic variability possibly explaining why only some women develop complications (25). While some women may sustain tubal damage after a single infection, others only develop complications after repeated or chronic infections (26). Several differences may also influence immune response, symptom severity, and infection persistence (27).

No significant differences were found between the groups regarding age, BMI, gestational age, or parity ($P \geq 0.05$), aligning with findings from Abdullateef et al. (28), who reported no significant difference in gestational age between EP and control groups.

CONCLUSION

Significantly higher proportion of women diagnosed with tubal ectopic pregnancy tested positive for anti-chlamydial antibodies compared to those with normal intrauterine pregnancies. Furthermore, the mean antibody titer was notably elevated in the EP group, suggesting a potential role for anti-chlamydial antibody levels as a predictive marker for ectopic pregnancy. This association remained significant regardless of the patients' age, gestational age, or parity. The predictive value of the antibody titer may be particularly useful in women presenting with non-specific symptoms such as chronic lower abdominal pain or vaginal spotting, thereby aiding in the early identification and management of ectopic pregnancies.

RECOMMENDATION

It is recommended that future research includes larger clinical trials involving a greater number of participants and a longer study duration to further investigate key aspects of Chlamydia trachomatis infection and its relationship with ectopic pregnancy. Specifically, such studies should aim to evaluate the predictive value of anti-chlamydial antibody levels in identifying women at risk of ectopic pregnancy. Additionally, assessing whether a combined diagnostic approach using both PCR and ELISA methods offers superior accuracy in detecting active or past infections would be valuable for guiding clinical management.

Moreover, routine screening for *Chlamydia trachomatis* is advised, particularly for sexually active women under the age of 25 and for women aged 25 and older who are at increased risk. Early detection and timely treatment in these groups can help prevent the long-term reproductive complications associated with undiagnosed or untreated infections, including ectopic pregnancy.

Ethical Clearance:

In accordance with the 2013 WMA Helsinki Declaration, all ethical aspects of this study were approved. Before enrolling the participants, an informed oral consent was obtained from their families as an ethical agreement. Additionally, approval from the hospital administrator was obtained.

Financial support and sponsorship: Nil.

Conflicts of interest: There are no conflicts of interest**.**

References

1. Jurkovic D, Knez J, Appiah A, Farahani L, Mavrelos D, Ross J. Surgical treatment of Cesarean scar ectopic pregnancy: efficacy and safety of ultrasound-guided suction curettage. Ultrasound Obstet Gynecol. 2016;47(4):511–7.
2. Talbot K, Simpson R, Price N, Jackson SR. Heterotopic pregnancy. J Obstet Gynaecol. 2011;31(1):7–12.
3. Tait L. Five cases of extra-uterine pregnancy operated upon at the time of rupture. Br Med J. 1884;1(1226):1250.
4. Barnhart K, Van Mello NM, Bourne T, Kirk E, Van Calster B, Bottomley C, et al. Pregnancy of unknown location: a consensus statement of nomenclature, definitions, and outcome. Fertil Steril. 2011;95(3):857–66.
5. Mangin D, Murdoch D, Wells JE, Coughlan E, Bagshaw S, Corwin P, et al. Chlamydia trachomatis testing sensitivity in midstream compared with first-void urine specimens. Ann Fam Med. 2012;10(1):50–3.
6. Machado AC, Guimaraes EM, Sakurai E, Fioravante FC, Amaral WN, Alves MF. High titers of Chlamydia trachomatis antibodies in Brazilian women with tubal occlusion or previous ectopic pregnancy. Infect Dis Obstet Gynecol. 2007;2007:24816.
7. Malik A, Jain S, Hakim S, Shukla I, Rizvi M. Chlamydia trachomatis infection and female infertility. Indian J Med Res. 2006;123(6):770.
8. O'Connell CM, Ferone ME. Chlamydia trachomatis genital infections. Microb Cell. 2016;3(9):390–403.
9. Peivandi S, Moslemizadeh N, Gharajeh S, Ajami A. The role of Chlamydia trachomatis IgG antibody testing in predicting tubal factor infertility in Northern Iran. Int J Fertil Steril. 2009;3(3).

10. Surana A, Rastogi V, Nirwan PS. Association of the serum anti-chlamydial antibodies with tubal infertility. J Clin Diagn Res. 2012;6(10):1692–4.
11. Hoffman BL, Schorge JO, Bradshaw KD, Halvorson LM, Schaffer JI, Corton MM. Williams Gynecology. 3rd ed. New York: McGraw-Hill Education; 2016.
12. Marion LL, Meeks GR. Ectopic pregnancy: history, incidence, epidemiology, and risk factors. Clin Obstet Gynecol. 2012;55(2):376–86.
13. Agholor K, Omo-Aghoja L, Okonofua F. Association of anti-Chlamydia antibodies with ectopic pregnancy in Benin city, Nigeria: a case-control study. Afr Health Sci. 2013;13(2):430–40.
14. World Health Organization. Body Mass Index [Internet]. 2019 [cited 2024 Apr 17]. Available from: www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi
15. Selo-Ojeme D, Onwude J, Onwudiegwu U. Autotransfusion for ruptured ectopic pregnancy. Int J Gynaecol Obstet. 2003;80(2):103–10.
16. Trabert B, Holt VL, Yu O, Van Den Eeden SK, Scholes D. Population-based ectopic pregnancy trends, 1993–2007. Am J Prev Med. 2011;40(5):556–60.
17. Felemban A, Sammour A, Tulandi T. Serum vascular endothelial growth factor as a possible marker for early ectopic pregnancy. Hum Reprod. 2002;17(2):490–2.
18. Horner PJ, Wills GS, Reynolds R, Johnson AM, Muir DA, Winston A, et al. Effect of time since exposure to Chlamydia trachomatis on chlamydia antibody detection in women: a cross-sectional study. Sex Transm Infect. 2013;89(5):398–403.
19. Akande VA, Hunt LP, Cahill DJ, Caul EO, Ford WC, Jenkins JM. Tubal damage in infertile women: prediction using chlamydia serology. Hum Reprod. 2003;18(9):1841–7.
20. Rantsi T, Joki-Korpela P, Wikström E, Öhman H, Bloigu A, Lehtinen M, et al. Population-based study of prediagnostic antibodies to Chlamydia trachomatis in relation to adverse pregnancy outcome. Sex Transm Dis. 2016;43(6):382–7.

21. Agholor K, Omo-Aghoja L, Okonofua F. Association of anti-Chlamydia antibodies with ectopic pregnancy in Benin city, Nigeria: a case-control study. Afr Health Sci. 2013;13(2):430–40.
22. Okunlola M, Okonikoko K, Adeyemi A, Adeyeni-Doro F. Chlamydia serology and ectopic pregnancy in Ibadin. Niger Med Pract. 2009;55(1–2).
23. Bokhari H. Anti-Chlamydial Antibodies in Women with Ectopic Pregnancy. J Rawalpindi Med Coll. 2017;21(3):215–8.
24. Darville T, Hiltke T. Pathogenesis of genital tract disease due to Chlamydia trachomatis. J Infect Dis. 2010;201(Suppl 2):S114–25.
25. Öhman H, Tiitinen A, Halttunen M, Paavonen J, Surcel HM. Cytokine gene polymorphism and Chlamydia trachomatis-specific immune responses. Hum Immunol. 2011;72(3):278–82.
26. Haggerty CL, Gottlieb SL, Taylor BD, Low N, Xu F, Ness RB. Risk of sequelae after Chlamydia trachomatis genital infection in women. J Infect Dis. 2010;201(Suppl 2):S134–55.
27. Menon S, Timms P, Allan JA, Alexander K, Rombauts L, Horner P, et al. Human and pathogen factors associated with Chlamydia trachomatis-related infertility in women. Hum Reprod Update. 2015;28(4):969–85.
28. Abdullateef HA. Serum Creatine Kinase and its Isoenzyme CK-MB in the Prediction of Tubal Ectopic Pregnancy. Iraqi J Med Sci. 2013;11(2):170–5.