

ELFA and IFAT Techniques to Detect Chlamydial Infections in Baghdad Women and Its Effect on the Immunoglobulins Level

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Summary:

Background: *Chlamydia trachomatis* is one of the most common human pathogens and considered as one of the causative agents of STDs. This organism cause acute and recurrent pelvic infections and infertility.

Patients and Methods: Two hundred and seventy three females were included in the present study, attending infertility department, AL-Elwiya hospital, AL-Jarah private hospital, central public health laboratory and STDs clinic to whom IFAT, ELAF and immunoglobulins concentration were done.

Results: Females were divided into three age groups <20; 20-39 and ≥40 years. Single and repeated abortions were 44.9%, 55.1% respectively. Primary and secondary infertility were 55.6% and 44.4%. Higher abortions rate were in the age group 20-39 years which represents 31.5% and 41.6% in single and repeated abortions. Primary and secondary infertility were high in the same age group which represents 42.6% and 33.3% of the total investigated females, also the multipartners within the same age group constitutes 56.7%. Chlamydial infections detected by IFAT technique constitute 12.8% which were represented as 14.6%, 11.1%, 36.7% and 5% in abortions, infertility, multipartners and fertility with no abortion groups respectively. While by ELFA technique the percentages were 12.4%, 9.3%, 30% and 4% in the same mentioned groups. Immunoglobulins mean value in females with chlamydial infections were as follows IgG = 2102.1 mg/dl; IgA = 317.9 mg/dl and IgM = 272.5 mg/dl. Which were more than the normal values of the immunoglobulins.

Conclusions: Chlamydial infections were distributed largely among multipartners than other aborted females. High abortions were noted in age group 20-39 years. IFAT technique was more reliable than ELFA technique to detect chlamydial infections.

IgG and IgM concentrations were higher than normal concentrations, while IgA remains normal.

Keywords: *Chlamydia*, IFAT, ELFA, Abortions, Immunoglobulins, STDs, Infertility, Multipartners.

Introduction:

Sexually transmitted diseases (STDs) were frequently encountered in family planning clinics and specially in certain high risk groups such as multipartner and couples, when one or both members have other sexual partner. The world health organization (WHO) has estimated that over 330 million people were infected with STDs, including syphilis, gonorrhoea, trichomoniasis and chlamydial infections during 1995 (1). In the developing countries half a million pregnant women die each year suffering from these infections (2). *Chlamydia trachomatis* is one of the most common human pathogens, second only to *Trichomonas vaginalis* as a causative agent of STDs (3). The major adverse impact of *Chlamydia trachomatis* infection in pregnant women is perinatal transmission of Chlamydia to neonate delivered through an infected

birth canal, which has little effect on prematurity, premature rupture of membranes and low birth infants. This organism cause acute and recurrent pelvic infections and infertility (4). So this study deals with two serological tests (ELFA and IFAT) to detect chlamydial infections in Baghdad women, with the estimation of immunoglobulins value (IgG, IgM, IgA) in these women.

Patients and Methods:

Patients: The study was carried out on 273 females attending general gynecological, obstetric, family planning and STDs clinic during the period from the first of August 1999 to the end of June 2000. It includes 173 females attending the obstetric and gynecological departments in three major hospitals:

1. Infertility department / AL-Elwiya hospital.
 2. AL-Jarah private hospital.
 3. Central public health laboratory and STDs clinic.
- The females were divided into three major groups, the first group (no. = 89) was females with history of

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abortions (40 single abortions and 49 repeated abortions).

The second group (no. = 54) was infertile females, which was separated into primary infertility (no. = 30) and secondary infertility (no. = 24), while the third group (no. = 30) included multipartner females.

Also one hundred fertile females with no abortions, gynecological or obstetric problems which were attending:

1. Family planning unit / AL-Elwiya hospital.
 2. Family planning unit / Baghdad teaching hospital,
- were included in this study. The women throughout the study were of different ages ranged from (16-45) years.

Females were examined by gynecologist. Endocervical swab and blood sample were collected from each female.

Antigen detection for Chlamydia by ELFA technique: Enzyme linked fluorescent assay (ELFA) is an automated quantitative test to detect chlamydial antigens in the endocervical swabs.

The technique was carried according to manufacturer instructions by using VIDAS instrument and VIDAS Chlamydia Assay (CHL) provided from Bio-Merieux Company (France).

Blood samples: A peripheral venous blood samples (5 ml) were taken from each female. The serum was separated by centrifugation and was used for IFAT technique and detection of immunoglobulins.

IFAT technique for detection of Chlamydia: Indirect immunofluorescent antibody technique was accomplished according to (5) by using females serum.

Quantitative determination of immunoglobulins:

Single radial immunodiffusion (RID) kit provided from Sanofi (France) was used, which is intended for the quantitative estimation of human immunoglobulins G, A and M. the diameter of endpoint ring was measured by using micrometer (IgG, IgA after 48 hrs. and IgM after 72 hrs.) and was converted to the concentration value by using special tables which represents quantitative determinations of immunoglobulins G, A. and M (6).

Results:

Females were distributed according to their ages into three categories (<20; 20-39 and ≥40 years) which were suffered from problems in bearing or ability to bear. The total aborted women (89) were divided into single abortion group (44.9%) and repeated abortions group (55.1%).

The infertile females group (54) was divided into primary infertility group (55.6%) and secondary infertility group (44.4%) as illustrated in table-1. the multipartner females group (30) showed high accumulations in the age category 20-39 years (56.7%)

the aborted and infertile groups were highly accumulated in the same previous age group.

Table-1: the incidence of abortions, infertility and the multipartners divided according to their ages.

Age categories (years)	Abortions		Infertility		Multipartners
	Single no. (%)	Repeated no. (%)	Primary no. (%)	Secondary no. (%)	
<20	7 (7.9)	8 (9)	2 (3.9)	5 (9.3)	9 (30)
20-39	28 (31.5)	37 (41.6)	23 (42.6)	18 (33.3)	17 (56.7)
≥40	5 (5.6)	4 (4.5)	5 (9.3)	1 (1.9)	4 (13.3)
Total (%)	40 (44.9)	49 (55.1)	30 (55.6)	24 (44.4)	30 (100)
Final total (%)	89 (100)		54 (100)		

The prevalence of chlamydial infections in 273 females were showed in table-2 which represents 35 (12.8%) by using IFAT techniques. Chlamydial infections were highly detected within multipartner females (36.7%), followed by aborted (14.6%), infertile (11.1%) and fertile with no abortions (5%).

Table-2: Results of IFAT technique for each females group.

Females group	Total no.	Positive results
		No. (%)
Abortions	89	13 (14.6)
Infertility	54	6 (11.1)
Multipartners	30	11 (36.7)
Fertile with no abortions	100	5 (5)
Total	273	35 (12.8)

The results of ELFA and IFAT techniques for detection of chlamydial infections distributed according to age categories were shown in table-3, which showed that the positive results constitutes 8.2% and 9.9% by using ELFA and IFAT techniques

respectively. The age category 20-39 years showed high accumulations for the positive results, since it were 5.8% and 7% by using ELFA and AIFAT techniques respectively.

Table-3: Results of ELFA and IFAT techniques for detection of chlamydial infections distributed according to age categories.

Age Categories (years)	ELFA technique		IFAT technique	
	+ve (%)	-ve (%)	+ve (%)	-ve (%)
<20	5 (2)	24 (9.9)	6 (2.5)	23 (9.5)
20-39	14 (5.8)	172 (70.8)	17 (7)	169 (69.5)
≥40	1 (0.4)	27 (11.1)	1 (0.4)	27 (11.1)
Total	20 (8.2)	223 (91.8)	24 (9.9)	219 (90.1)

Table-4 illustrates the incidence of chlamydial infections for each females group by using ELFA and IFAT techniques. It was noted that IFAT technique was more sensitive (14.6%) than ELFA (12.4%) to detect the positive cases within the aborted group, since it was more clear in cases of single abortion (9% and 11.2%) than in repeated abortions (3.4%). Also it was more clear in the second trimester (10.1%) than that in the first trimester (2.2% and 4.5%).

Chlamydial infections in infertility group reached (9.3%) and (11.1%) by using ELFA and IFAT techniques respectively. It was important to know that (30%) and (36.7%) of the multipartner group was infected by *Chlamydia* by using ELFA and IFAT techniques respectively.

The fertile group with no abortions showed the lowest percentages of chlamydial infections (4% and 5%) by using the above techniques. Immunoprecipitin ring diameters of Igs were measured and converted to concentrations by using single radial immunodiffusion test. Igs in patients with chlamydial infections were compared with the normal range of Igs as showed in table-5. The different age categories showed high values of IgG than normal values, since it reached 2222.9; 1951.3 and 2132 mg/dl respectively. There were no changes appeared in IgA mean values, but more differences appeared in IgM mean values which reached 308, 279.4 and 230 mg/dl respectively which were higher than the normal values. IgG and IgM means were high in the age categories 20-39 and ≥40 years.

In healthy women group who were fertile women with

no abortions, no differences of Igs mean values seen in all age categories, just IgM mean values in the age category 20-39 years which reached 195.1 mg/dl. IgG and IgM mean values were high in age category 20-39 years followed by age category ≥40, while IgA mean values were high in age category ≥40 which were more than other age categories.

Table-4: The incidence of chlamydial infections by using ELFA and IFAT techniques for each

Females group	Total no.	ELFA technique		IFAT technique	
		+ve (%)	-ve (%)	+ve (%)	-ve (%)
Abortion	89	11 (12.4)	78 (87.6)	13 (14.6)	76 (85.4)
-First trimester		2 (2.2)	37 (41.6)	4 (4.5)	35 (39.3)
-Second trimester		9 (10.1)	41 (46.1)	9 (10.1)	41 (46.1)
-Single		8 (9)	32 (36)	10 (11.2)	30 (33.7)
-Repeated		3 (3.4)	46 (51.7)	3 (3.4)	46 (51.7)
Infertility	54	5 (9.3)	49 (90.7)	6 (11.1)	48 (88.9)
-Primary		2 (3.7)	28 (31.5)	3 (5.6)	27 (30.3)
-Secondary		3 (5.6)	21 (23.6)	3 (5.6)	21 (23.6)
Multipartner	30	9 (30)	21 (70)	11 (36.7)	19 (63.3)
Fertility with no abortions	100	4 (4)	96 (96)	5 (5)	95 (95)

females group.

Table-5: Immunoglobulins mean values in patients with chlamydial infections compared with fertility group distributed according to age categories (years).

Age categories	Chlamydial infections			Fertility with no abortions		
	IgG	IgA	IgM	IgG	IgA	IgM
<20	2222.9	325.5	308	1252.8	152.8	161.8
20-39	1951.3	335.2	279.4	1604.6	245.6	195.1
≥40	2132	293	230	1512.8	282.2	183.4
Total mean	2102.1	317.9	272.5	1456.7	226.9	180.1

Normal immunoglobulins value (mg/dl)

IgG = 844-1912

IgA = 68-423

IgM = 50-196

Discussion:

The prescience of chlamydial antigens in the endocervical swabs were estimated by using ELFA technique and in serum specimens, by using IFAT technique. *Chlamydia* appeared as a bright yellow-green elementary bodies by using IFAT technique, which were easily demonstrated by using fluorescent microscope. The ration of chlamydial infections in the present study was 12.8% and it is nearest to that found in Saudi Arabia which was 10.8% (7).

Many of chlamydial infections are asymptomatic and thus go untreated. The infection is not only serve as a reservoir for further spread of infections, but it's sequel can be severe affecting reproduction, fertility, infant morbidity, mortality and its complications include salpingitis (8), tubal factor infertility and ectopic pregnancy (9).

Some researchers (10) reported that some of the maternal, fetal morbidity and mortality may be associated with both acute and past chlamydial infections. In infertile females, the ratio of infection with *Chlamydia trachomatis* reached 75% (11) and it was found that 44% of them were without gynecological disease and 87% of the infections were found in females with sexually transmitted diseases (STDs). Ectopic pregnancy due to this pathogen was noted in 40-50% of the total investigated cases (12).

The rate of infections in the present study was lower than that found in the developing countries because the infection was associated with many risk factors which include race, marital status and the presence of multiple sexual partners (13). IFAT technique was more sensitive than ELFA to detect the positive cases of chlamydial infections and this may be explained by the fact that IFAT technique estimate serum antibodies which might be raised due to other site of infection like in trachoma and Rieter's syndrome which stimulate the immune system to produce antibodies.

Rates of infection were varied worldwide, in Iraq it reached 9.6% in previous study (14), while in Egypt it reached 30% (15). Also high rates of infection were noted in the age group 20-39 years and this can be explained due to the high sexual activity during these ages. Some authors concluded that *Chlamydia* can be isolated more frequently from younger women (16, 17). The low prevalence of infections in older age groups suggests that such infections are often self-limiting or those women are sexually less active. Similar conclusion was suggested in Mexico (18). It was found that 57% of chlamydial infections were in ages less than 19 years, 29% in ages 20-24 years and only 13% in the ages >25, which may be explained by the fact that the tub partners were untreated from such infections (19). The incidence of infections in the second trimester was more than that in the first trimester. This result was in agreement with other study, which found that 30% of the infections were in the first trimester and 53.3% in the second trimester (20). The effect of maternal chlamydial infection on pregnancy outcome and prenatal complications such as preterm delivery, premature reapture of membrane and post partum fever remains controversial (4). Chlamydial infections were associated with 38.9% of the repeated abortion cases and only noted in 8.3% of the single abortion cases (21). It was reported that *Chlamydia trachomatis* infections were associated with reported abortions and the mechanism may

involve reaction of a latent chlamydial infection or an immune response to an epitope shared by chlamydial and fetus antigen (22). Chronic silent chlamydial infections may increase susceptibility to early pregnancy loss through immune system activation which might destroy the embryo or interfere with immune regulatory mechanism (22). The effect of chlamydial infections on the termination of pregnancy will be depends on the infecting inoculum, time of gestation and susceptibility of the host to infection (23). The early genital infection during gestation with high chlamydial inoculum can result in premature termination of pregnancy, while a low inoculum does not appear to affect the course of gestation (3). The fetus membranes were affected following the infection of the endometrium, it is possible that the direct damage to the fetus membranes might result from the infection, in combination with the endotoxin activity of the chlamydial lipopolysaccharides were significant factors in the premature termination of pregnancy (23). In the infertility group, it was noted that abnormal pregnancy occurrence and infertility observed was most likely due to an obstruction of the oviduct (24). It was reported that the infection of the genital tract with *Chlamydia trachomatis* represent one of the most common causes of women infertility (13). In Egypt it was found that 40% of infertility cases were infected with *Chlamydia trachomatis* (15). Other study showed that infertility despite of the positive antibody to *Chlamydia trachomatis*, none of the oviduct biopsy culture was positive for *Chlamydia trachomatis* (25). Some authors refered that 5% of fertile women with no abortions suffered from chlamydial infections (21). This ration was more than that recorded by others (26), since the ratio was 2.5% in women attending family planning clinics, while in Dublin the ratio was 12.5% (27). *Chlamydia trachomatis* is a common infective agent of the genital tract for women attending clinics for venereal diseases and these results were in agreement with other studies carried in USA (28), also in Dublin the incidence of infection was 18% (29). High rates of chlamydial infections (33.2%) were noted in ages less than 19 years, while in older ages the incidence was low (11.2%) (13). The risk of *Chlamydia trachomatis* infection is increase in ages less than 25 years, or in the presence of more than one sexual partner or lack of barrier contraceptive (30). STDs clinics in Ethiopia recorded that 32% of the investigated women were infected with *Chlamydia* (31). These findings support the concept that *Chlamydia trachomatis* is a pathogen in the genital tract and is sexually transmitted. The differences between the rates of infection among the different studies may be attributed to the variation in the sensitivity of the detection procedure, or may be related to the differences in the prevalence of chlamydial infections in various areas at different

times of the year, or the possibility of association of chlamydial infections with other STDs or may be due to the differences in the sample size.

The increase of IgG antibody levels may reflect past infection or exposure to chlamydial infection, while the increase of IgA levels may reflect persistent or chronic infections (32). The levels of *Chlamydia* antibodies were correlated well with the severity of tubal inflammation and with duration of lower abdominal pain, or these changes may be related to the recurrent abortions which is caused by chlamydial infections (15). In healthy fertile women with no abortions, no differences of Igs mean values were seen in all age categories, just for IgM mean values within age category 20-39 years were more than the normal range which may be explained that this age range represent more active age, that may be attributed to their sexual activity (33).

References:

- (1) Gerbase, A. C.; Rowley, J. T.; Hevmann, D. H. L.; Berkley, S. F.B. and Piot, P. (1998). Global prevalence and incidence estimates of selected curable STDs. *Sex Transm Infect.* 74 Suppl. 1:512-6.
- (2) Whitfield, C. R. (1995). *Obstetrics and Gynecology*. 5th. Ed. Blackwell Science, Great Britain.
- (3) Pal, S.; Hui, W.; Peterson, E. M. and Luis, M. (1998). Factors influencing the incidence of infertility in a mouse model of *Chlamydia trachomatis* ascending genital tract infection. *J. Med. Microbiol.* 47:599-605.
- (4) Sweet, R.L.; Landers, D. V.; Walker, C. and Schachter, J. (1987). *Chlamydia trachomatis* infection and pregnancy outcome. *Am. J. Obstet. Gynecol.* 156(4):824-33.
- (5) Gilbert, G. L. (1996). *Chlamydia*. In *Practical Medical Microbiol.* Edited by Collee, J. G.; Fraser, A. G.; Marmion. New York. PP:621-33.
- (6) Mancini, G.; Carbonara, A. O. and Heremans, J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*. 2:235-254.
- (7) Qadri, M. S. H.; Akhtar, J. and Ignacio, K. (1993). Incidence of *Chlamydia* infections in a large metropolitan hospital in Saudi Arabia. *Saudi Med. J.*, 14:152-5.
- (8) Sierra, M. F.; Clarke, L. M. and Boyle, J. F. (1988). The laboratory diagnosis of chlamydial infection. *Lab. Med.*, 19:311-4.
- (9) Svensson, L.; Mardh, R. A.; Ahlgren, M. and Nordenskjöld, F. (1985). Ectopic pregnancy and antibodies to *Chlamydia trachomatis*. *J. Fertil. Steril.* 44:313-16.
- (10) Gencay, M.; Puolakkainen, M. and Wackström, T. (1997). *Chlamydia trachomatis* detected in human placenta. *J. Clin. Pathol.* 50:852-5.
- (11) Degen, K. W.; Distler, W.; Graf, M. and Kreck, T. (1990). Cultural and serologic *Chlamydia* detection in diagnosis of sterility and increased risk of infection. *Geburtshilf Frauenheilkd.* 50(5):371-4.
- (12) Marana, R.; Sanna, A. and Lucisano, A. (1990). High prevalence of silent *Chlamydia* colonization of the tubal mucosa in infertile women. *J. fertility and sterility*. 53(2):354-7.
- (13) Aral, S. O.; Hughes, J. P. and Stoner, B. (1999). Sexual mixing patterns in the spread of gonococcal and chlamydial infections, 89(6):825-33.
- (14) Mohammed, N. S. R.; Al-Omari, W. R. Al-Ani and Ahmad, T. A. (1990). Interrelationship of *Chlamydia* subgroup and other pathogens in the female genital tract. *J. Fac. Med.* 32(3):339-43.
- (15) Diab, K. M.; Abd-El-Maeboud, K. H.; Abou-Loz, M. M. and Mokhtar A. M. (1993). Gonococcal and chlamydial antibodies in Egyptian with ectopic pregnancy. *New Egyptian J. Med.* 8(4):1006-10.
- (16) Stamm, W. E. (1999). *Chlamydia trachomatis* infections: Progress and Problems. *J. Infect. Dis.* 179. suppl (2):380-3.
- (17) Chernesky, M. A.; Mahony, J. B.; Castriciano, S.; Mores, M. (1986). Detection of *Chlamydia trachomatis* antigens by enzyme immunoassay and immunofluorescence in genital specimens from symptomatic and asymptomatic men and women. *J. Infect. Dis.* 154:141-8.
- (18) Acosta Cazares, B.; Mayal, R. and Delapena, E. J. (1996). Prevalence and risk factors for *Chlamydia trachomatis* infection in low in comp rural and suburban populations of Mexico. *J. Sex. Trans. Dis.* 23(4):283-8.
- (19) Foglia, G.; Rhodes, P.; Goldberg, M. and Louis, M. E. (1999). Completeness of and duration of time before treatment after screening women for *Chlamydia trachomatis* infections. 26(8):421-425.
- (20) Bassim, H. H. and El-Zeneny, H. (1993). *Chlamydia trachomatis* cervical infection among cases of early abortion. *Egyptian J. Medical Laboratory Sciences*. 2(3):249-55.
- (21) Amer, A. A.; Zaitoun, A. M. and Azab, M. M. (1992). *Chlamydia trachomatis* infection in women with recurrent spontaneous abortion: a serological study. *New Egyptian Journal of medicine*, 7(4):804-5.
- (22) Witkin, S. S. and Ledger, W. J. (1992). Antibodies to *Chlamydia trachomatis* in sera of women with recurrent spontaneous abortions. *Am. J. Obstet. Gynecol.* 167:135.
- (23) Spiliopoulou, D.; Psarrou, E.; Rodolakis, A. and Vretou, E. (1992). Protection of mice infected with *Chlamydia trachomatis* from abortion by passive

immunization, P. 103. In P. A. Mardh, M.; Laplaca by passive and ward proceedings of the European Society for Chlamydia research. Uppsala University Center for STD Research, Uppsala, Sweden.

(24) Tuffrey, M.; Alexander, F.; Inman, D. and Ward, M. E. (1990). Correlation of infertility with altered tubal morphology and function in mice with salpingitis induced by a human genital-tract isolate of *Chlamydia trachomatis*. *J. Reprod. Fertil.* 88:295-305.

(25) Brunham, R. G.; Binns, B.; Mc-Doweli, J. and Paraskevas, M. (1986). *Chlamydia trachomatis* infection in women with ectopic pregnancy. *J. Obstet and Gynecol.* 67:722.

(26) Abdullah, M. (1999). "Epidemiological characteristics of female patients with genital *Chlamydia* infection attending the department of gynecology and obstetric of Baghdad teaching hospital". M. Sc. Thesis. College of Baghdad Medicine.

(27) Mohammad, N. R. S. (1981). Incidence of chlamydial infection in cervical swabs of women attending special clinics. M. Sc. Thesis. University College Dublin.

(28) Kinghorn, G. R. and Waugh, m. a. (1981). Oral contraceptive use and prevalence of infection with *Chlamydia trachomatis* in women. *Br. J. Vener. Dis.* 57:187-90.

(29) Mohammed, N. R. S.; Quoid, G.; Hillary, I. B. and verling, W. H. (1984). Incidence of *Chlamydia trachomatis* infection in patients attending a Dublin Venereal Disease Clinic. *Irish medical Journal.* 77(4):102-4.

(30) Horner, P. G. and Caul, E. O. (1999). National guideline for the management of *Chlamydia trachomatis* genital tract infection. *J. S.T.D.* 75. Suppl (1):4-8.

(31) Bashir, S. A. (1986). Chlamydial genital infection. *Saudi Med. J.* 7:484-9.

(32) Patiel, O.; Kark, J. D.; Leinonen, M. and Saikku, P. (1995). High prevalence of antibodies to *Chlamydia pneumoniae*; determinants of IgG and IgA seropositivity among Jerusalem residents. *J. Epidemiol Infect.* 114:465-73.

(33) Ryan, G. M.; Abdalla, T. N.; Menealy, G. and Baselskiv, S. D. (1990). *Chlamydia trachomatis* infection in pregnancy. *Am. J. Obstet. Gynecol.* 187:34-9.