The detection of Chlamydia Trachomatis Antigen in cervical secretions and serum antibodies in infertile females undergoing ICSI and its impact on pregnancy success.

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Abstract: Background: Chlamydia vaginosis is a commonly reported bacterial infection, with an incidence that varies according to population. Some researchers have studied the association of such condition and infertility. In our study we aimed at evaluation of the incidence of cervical Chlamydia trachomatis infection in infertile women undergoing assisted reproductive techniques & its impact on its success to achieve pregnancy. **Methods:** A study group of 150 infertile females attending infertility centers for ICSI and a control group of 150 multiparus females attending outpatient clinics for IUD introduction have been included. Endocervical swabs from cases and controls have been examined for chlamydial antigen using immunochromatography. Also sera were examined for IgG and IgM for Chlamydia trachomatis in both groups. **Results:** 12 positive cases for serum Ig G in study group (8.0%) and 16 in controls (10.67%) has been revealed. While serum IgM was found in 4 study cases (2.7%), with no positives in the controls. Regarding chlamydia antigen detection in endocervical swab, there was 6 positive study group cases (4.2%), while no cases were positive in controls. **Conclusion:** our study reports a very low prevalence rate of *Chlamydia trachomatis* infection in Egyptian females, which minimizes its role as cause of infertility in Egyptian population and subsequently its impact on success of ICSI is not much expressed. Cultural impact on sexual life style in Egyptian population could justify our findings.

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Key words: Chalmydia, antigen, antibody

1. Introduction

Chlamydial vaginosis is a commonly reported bacterial infection specifically in developed countries. The causative agent of the condition is chlamydia trachomatis (Stamm, 1999), with an incidence of infection that ranges from 8.6% up to 40% according to population with a reported higher association with infertility (Woods, 2003). C. trachomatis being the causative agent of a widespread sexually transmitted disease can lead to urethritis, cervicitis pelvic inflammatory disease (PID), ectopic pregnancy and tubal factor infertility in females (Mayaud, 2001). Persistence, chronicity and subclinical presentation are the common characters of the C. trachomatis infection (Schachter, 1999). It has been suggested that impaired ovarian function and low ovarian response to ovulation induction are associated with C. trachomatis infection. (Malik et al, 2006) In females, infection usually starts in cervix and urethra with an abnormal vaginal discharge or a burning sensation in micturition. Then it usually spreads to the fallopian tubes and uterus and can progress to pelvic inflammatory disease. Still, silent infections are prevalent. (Stephen, 1999)

Earlier studies have shown that pathogenesis of the disease was not only induced by

the infectious agent but also due to immune response to infected tissues. (Paavonen et al 1999).

Various diagnostic assays are available for Chlamydia infection . Previously, cell culture of cervical, vaginal swab and urine was the main tool for objective diagnosis of the case. Immunological tests by detection of antibodies (IgG, IgM) were also available. However, it is reported that serum antibody was not always consistent with infection (Brunham et al 1983). Molecular methods such as PCR are considered as reliable methods for diagnosis nevertheless, they are technically demanding and with low cost effectiveness in case of screening. Detection of chlamydia antigen in different samples have been reported to be high sensitive and specific and rapid kits are available commercially with simplicity of usage and low cost. (Machado, 2001) The aim of this study is to evaluate the incidence of cervical *Chlamvdia trachomatis* infection in infertile women undergoing assisted reproductive techniques & its impact on its success to achieve pregnancy.

2. Subjects and methods.

Study group: Demales attending fertility centers in Alexandria seeking ICSI (intracytoplasmic sperm injection) for treatment of infertility.

Control group comprising multiparus

females attending the contraception clinics for IUD introduction was included for comparison. Cases included in this study have been informed and consented for this study. Criteria for selection of cases are summarized as follows:

Inclusion criteria :

- 1- Age range between 20 and 40 years.
- 2- Duration of infertility more than 3 years.

Exclusion criteria:

1- Systemic diseases such as diabetes, hypertension, renal disease & systemic lupus

2- Local disease such as fibroid, uterine anomalies as uterine septum & adenomyosis diagnosed by 3 D ultrasound or hystrosalpingography.

3- Moderate to severe endometriosis

4- Severe male factor of the male partner (count of sperm <5 millions /ml, abnormal forms of sperms more than 90% and motility less than 10%).

Cases included in this study have been subjected to:

- 1. Collection of endocervical swab: after cleaning of cervical canal from mucus, a sterile swab has been rubbed for 15 seconds to the cervical canal after passing squamocolumnar juction (ie: tip of the swab is not visible). Chlamydia Antigen in the collected samples was detected using rapid Immunochromatographic assay using commercially available kits according to manufacturer's instructions (Prechekbio, Invitro diagnostics, Korea). (Woolley et al, 1997) Results have been considered positive in case of presence of two distinct colored lines: one in control line region & another one in the test region. Negative result was considered in case of detection of only one colored line in the control region.
- 2. Withdrawal of 2 ml venous blood and serum was separated for detection of Chlamydia antibodies (IgM, IgG) using ELISA. The Chlamydia trachomatis antigen which is coated on the plates is comprised of elementary bodies. (*IBL International GmbH*, USA). (Herrman et al, 1991, Simms et al, 1987, Thomas et al 2000).
- 3. Follow up for pregnancy results of ICSI: (Intracytoplasmic sperm injection) by estimating B-hCG and ultrasound visualization of fetal heart beats.

Statistical analysis:

The Data was collected and entered into the personal computer. Statistical analysis was done using Statistical Package for Social Sciences (SPSS/version 15) software. The statistical test used as follow: Arthematic mean, standard deviation, for categorized parameters, chi square test was used while for numerical data, for two groups non parametric data U-test was used, while for two groups (parametric data), t-test was used. The level of significant was 0.05.

3. Results:

This study was carried out on 300 married females. Study group included 150 females attending fertility clinics for management of infertility, while a group comprising 150 multiparus females attending the outpatient clinic for IUD introduction for contraception were included as control. The age range in the study group was 20 - 36 years with a mean of 29.05±4.18 and in control group it was 27 -35 years with a mean of 30.21±2.917. There was no significant difference between the two studied groups (p>0.05). No difference in the body mass index between the study and control groups was observed which ranged from 22.1 to 40 (mean 27.15±3.826) and 21.2 - 39 (mean 28.53±2.917) in the study and control groups respectively (P=0.166) .The duration of infertility in the study group was 3 - 10 years with mean of 6.07 ± 2.461 . We report that there were 12 positive cases for serum Ig G in the study group (8.0%) and 16 positive cases in control group (10.67%). Further, in the study group, serum IgM was found only in 4 cases (2.7%), while no one in the control group were positive for serum IgM. There was no statistical significant difference between the two studied groups with respect to serum IgM and IgG. Chlamydia antigen was detected in 6 cases (4.2%) in the study group, while no positives were detected in the control group. There was no statistical significant difference between the study and control groups regarding chlamydial antigen positivity.

Table (1): Comparison between study and control groups regarding laboratory assays used to diagnose Chlamydial infection.

Assay	Patients "n=150"		Control "n=150"		р
	No.	%	No.	%	
Chlamydial Ag positive in endocervical swabs	6	4.2	0	0.0	0.36
Serum IgM positive	4	2.7	0	0.0	0.618
Serum Ig G Positive	12	8.0	16	20.0	0.086

There was no difference between the negative and positive cases of the study group concerning the age which ranged from 20 - 36 (mean 29.01±4.155) years in negative cases while in positive cases it ranged from 23 - 35 years (mean 30.17 ± 5.115) years, (p>0.05). The body mass index in the negative cases ranged from 22.1 - 40.0 (mean 27.09 ± 3.867), while in the positive it ranged from 25.0-32 (mean 28.67 ± 2.422). There was no statistical

significant difference between the two studied groups regarding body mass index (p > 0.05). The duration of marriage in the negative cases ranged from 3-10 years (mean 6.04 ± 2.452), while in the positive cases it ranged from 3 - 10 years (mean 6.83 ± 2.787) with no statistical significant difference between the two studied groups (p>0.05). In order to determine whether other conditions might be associated with infertility in the study group, a wide variety of parameters were considered. It was found that the most common cause (30.7%) for infertility was polycystic ovaries (PCO), while the travelling of husband was the least (6.7%). It was also found that there were 8 cases with unexplained cause (Table 2).

Table (2): Distribution of the studied cases regarding other conditions associated with infertility.

Cause of infertility	Number	Percent
Endometriosis	16	10.7
Male factor*	38	25.3
PCO	46	30.7
Travelling husband	10	6.7
Tubal factor	32	21.3
Unexplained	8	5.3
Total	150	100.0

^{*} Male factor 5-10 million in count, motility10-40% and abnormality 60-80%.

Correlation between chlamydial antigen results obtained by endocervical swabs and serum IgG detection, it was found that 11 cases (7.6%) of the negative for chlamydia antigen were positive for serum IgG, whereas in cases positive for chlamydia antigen only 1 case was positive for serum IgG. There was no statistical significant difference between the negative and the positive Chlamydia antigen cases in relation to serum IgG. It was also found that there were no cases that were positive for IgM in the samples that were negative for chlamydia antigen In cases positive cases for chlamdia antigen 4 cases were positive for a positive serum IgM. There was no statistical significant difference between the negative and the positive chlamydia antigen in relation to serum IgM.

Table (3): Relation between Chlamydia antigen detection and serum IgM in study group cases.

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	Chlam (Ag) in endocervical swab				
	Negative"n=144"		Positive"n=6"		р
	No.	%	No.	%	
IgM					0.15
Negative	144	100	2	33.3	0.15
Positive	0	0	4	66.7	2
IgG					0.20
Negative	133	92.4	5	83.3	0.39
Positive	11	7.6	1	16.7	9

On analyzing the pregnancy outcome of the study group cases after ICSI, it was found that there was 47 cases were positive for pregnancy test (quantitative B-hCG (31.3%). It was found that 2 cases were positive (33.33%) in cases positive for chlamydia antigen. It was found that 45 cases were positive in patients negative for chlamydia antigen (31.3%), There was no statistical significance difference in B-hCG results with respect to Chlamydia antigen positivity.

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Pregnancy	Study group cases "n=150"		Positive for chlamydia Ag		Negative for chlamydia Ag	
	Ν	%	N=6		N=144	
Negative	103	68.7	4	66.67%	99	68.7%
Positive	47	31.3	2	33.33%	45	31.3%
р			0.415			

Table (4): Pregnancy after ICSI in the study group

4. Discussion:

Underreporting is an obstacle in assessing the total burden of Chlamydia infection in the Middle East. Many factors contribute to this underreporting. Some of them include the culture of the population that judge sexually transmitted diseases as a stigma and the financial problem of high cost and technically demanding diagnostic assays for the medical condition that makes these diagnostics not available in routine labs. A previous study reported that Chlamydia trachomatis prevalence was found to be 5% in Cairo. (Sadek et al, 1991)

Various methods are available for the diagnosis of Chlamydia infection such as cell culture of cervical-vaginal swab, (Marrazzo et al 1997) serological testing by detection of specific antibodies: IgM for recent active infection, IgG for previous or chronic infection. Inspite of being useful as diagnostic tool for ocular or respiratory infection. studies reported that the production of the specific antibodies for chlamydia in case of genital tract infection is not consistent and may be absent in some cases (Simms et al 1991). Detection of Chlamydia antigen in different samples according to site of infection has been widely used in the developed countries for screening of cases for its simplicity, cheapness and rapidity. (Neuer et al, 2004, Johnson et al, 2002, Lossick et al 1990). Detection of the nucleic acid of chlamydia organism via Polymerase chain reaction (PCR) is still a gold standard for diagnosis though it is expensive and technically demanding. (Nelson et al, 2001, Rosenwaks et al, 1995)

Many researchers investigated the role of different microbial agents in infertility and the impact of their existence on outcome of assisted reproductive techniques. In this study we aimed at assessing the incidence of chlamydia trachomatis infection in infertile females. As regards the incidence of positive serum IgG in our study, it was found that positive cases in infertile females (8.0%) where lower than in control (10.67%), however this difference was not significant.Different countries reported widely variable incidence of chlamydial genital infection. in Australia, incidence reported of Chlamydia trachomatis antibodies in patients attending infertility clinics was at minimum 30%. (Torode et al, 1994) Sharara et al. in 1997 in the United States found that the prevalence of elevated serum chlamydial IgG Ab in female patients presenting for IVF was (55.2%) which was higher than in the general population. (Sharara et al 1997). The lower incidence of positive chlamydial IgG in our study compared to studies conducted in western countries could be attributed to lower frequency of predisposing factors of chlamydial infection including early sexual life and female multiple partners.

Regarding serum IgM the comparison between the two studied groups showed that there were only 4 cases (2.7%) in the study group positive for serum chlamydial IgM, while no one in the control group, still with no significant difference. A study conducted by Annika Idahl, in Sweden, reported higher prevelance of positive serum chlamydial IgM which was about 13% (Idahl, 2009).

In the present study, the higher incidence of positive chlamydial IgG in comparaison to IgM could be attributed to IgM antibody being reflecting acute infection while infertile patients have usually chronic infection, also positive IgG result does not necessarily indicate a genital chlamydia trachomatis infection, as it could be an infection elsewhere in the body. Regarding prevelance of chlamydia (Ag) among study group cases, a low prevalence 4.2% has been revealed, while no positive cases were detected in control group. Still, due to low prevalence in study group there is no significant difference with controls.

Results obtained by <u>Osser S</u> et al. (study conducted in Sweeden on 121 infertile female with tubal factor (Osser et al, 1990). Other researchers reported no significant impact on ICSI outcome (Tasdemir et al, 1994). On the other hand, Witken et al. study showed decrease in embryo characterization in positive cases for chlamydia (Ag), this was justified by heat shock protein induced by inflammatory reaction in response to infection that may impair embryo implantation and/or facilitates immune rejection after uterine transfer of in vitro fertilized embryos (Witkin et al, 1994).

In the present study, the association of chlamydial Ag in endocervical swab and serum IgG, it was found that there was 11 cases had negative chlamydial antigen while their serum had positive serum IgG, whereas there was only one case had

positive chlamydial (Ag) in endocervical swab show positive serum IgG. There was no significant association between prevelance of chlamydial (Ag) in cases and serum IgG. The higher rate of positive chlamydial IgG may be explained by the chronicity of infection in the patients. It was found that all cases negative for chlamydial antigen results had no positive serum IgM, whereas in the 6 positive cases for the antigen only 4 cases showed positive serum IgM that could be considered as acute chlamydial infection. Many previous studies that had assessed the effect of chlamydia trachomatis infection on the success rate of assisted reproductive techniques, reported that pregnancy outcome of IVF was not affected with previous exposure to chlamydia trachomatis (Sharara et al 1997, Tasdemir et al 1994). Moreover, Acharya, et al study reveal although strong association of bacterial vaginosis and past chlamydial infection with tubal infertility but with no impact on in vitro fertilization success rates (Acharya et al 1999).

Our study reports a very low prevalence rate of Chlamydia trachomatis infection in Egyptian females, about 4.2%. This low prevalence limits the study of its association with success of ICSI cycles. Whereas in other countries with higher prevalence, impact of chlamydial infection on infertility is much expressed. Cultural and religious impact on sexual life style in Egyptian population could justify our findings. As a consequence of our findings, Chlamydia trachomatis genital infection can't justify raising prevalence of primary infertility in Egyptian population. It could be undertaken by the clinicians in such population in management of infertility.

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