

Full Length Research Paper

Screening for *Chlamydia trachomatis* infection among infertile women

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Accepted 21 July, 2015

Chlamydia trachomatis infection is a worldwide distributed sexually-transmitted infection that may lead to infertility. This study aims to report the prevalence of *Chlamydia trachomatis* infection among infertile women in Saudi Arabia. A *community-based* study, carried out at obstetrics and gynaecology clinic at Jazan General Hospital, Saudi Arabia. The study-group included 640 infertile women who aged between 18 and 40 years-old and attended the gynaecology clinic for infertility work-up throughout the one year of the study. The control-group include a randomized 100 fertile women who attended the obstetric clinic for routine antenatal-care. All recruited women were screened for *Chlamydia* infection by enzyme-linked immunosorbent assay (ELIZA) for detection of serum specific antibodies and then re-tested by McCoy cell culture technique. The prevalence of *Chlamydia trachomatis* infection among infertile women was high, 15.0%. The rate of chlamydia infection detected by ELIZA was 9.84% while it was 12.03% by the culture method ($p = 0.2443$). The high prevalence of *Chlamydia trachomatis* infection among infertile women demands a national screening programme for early detection. ELIZA is a simple alternative screening test to the culture method.

Key words: Chlamydia trachomatis, ELIZA, McCoy cell culture, infertility.

INTRODUCTION

Chlamydia trachomatis (serotypes D - K) are obligatory intracellular gram negative bacteria that primarily infect the female cervix, urethra and fallopian tubes. The majority of infection is asymptomatic and goes undetected with an increased risk of pelvic inflammatory disease (PID), the leading cause of ectopic pregnancy, tubal factor infertility⁽¹⁻³⁾ and chronic pelvic pains.

In Saudi Arabia, the incidence of sexually-transmitted infections (STI) is low, unparalleled to that in developed countries. Gonococcal and non-gonococcal urethritis and genital warts are frequently reported among Saudis, while acquired immune deficiency syndrome (AIDS), syphilis, and genital herpes are frequently reported among the non-Saudis.⁽⁴⁾

For diagnosis of chlamydia infection, cell culture of urogenital specimens is considered the ideal method, although few laboratories could offer this due to cost and lack of skills in cell-culture technique. Accurate results

depend on proper sample-taking, carrying, storage and interpretation. Although culture is 100% specific for chlamydia (*no false positive*), there is a growing observation that culture is not 100% sensitive. The combination of 100% specificity and the ability to detect viable organisms make culture is the standard for legal applications as sexual assault.

With the availability of more rapid assays during the 1980s, many laboratories started to use Enzyme-linked Immunosorbent Assay (ELISA) due to lesser demands of cost, skills, and time required for obtaining the results. However, these tests were less sensitive as detection of antibodies in a single serum sample is frequently found in absence of active infection.⁽⁵⁾ Despite difficulty of differentiating between previous and current infections, the presence of chlamydia specific antibody (IgA) is significantly associated with upper genital tract infection, particularly when the antibody titre is high.^(6,7) As the sen-

sensitivity of ELIZA test is low this can assist but cannot replace direct antigen detection or isolation of organism by the culture technique.⁽⁸⁾

Another widely-used rapid simple test is the Enzyme Immuno-assay (EIA). It does not require any sophisticated equipment and can be completed within 30 minutes. It is significantly less sensitive and specific than the laboratory-based tests. Reported sensitivities of rapid tests relative to culture range from 52-85% for endocervical swabs and its specificities are over 95%.⁽⁹⁻¹¹⁾ However, rapid tests should not be used in a low-prevalence population or for asymptomatic women due to the potential of false-positives. Its results should always be confirmed by a laboratory-test.

More recent and very sensitive Nucleic Acid Amplification Techniques (NAAT), such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) have been used for detection of chlamydia genetic material DNA in cervical and urethral samples.⁽¹²⁾

A further development is Nucleic Acid Hybridization (DNA-Probe) tests, which also detect chlamydia genetic material DNA. These tests are very accurate but are not as sensitive as the NAAT. Other diagnostic tests include; Transcription-mediated Amplification (TMA) which amplifies the ribosomal-RNA, Strand Displacement Amplification (SDA), and Direct Fluorescent Antibody (DFA) tests. Papanicolaou (PAP) smear should not be used for chlamydia screening as it has poor sensitivity and specificity.

Sweden is known to have the best chlamydia screening programme in the world.⁽¹³⁾ In the United States, center for disease control and prevention (CDCP) has supported screening programme since 1988.⁽¹⁴⁾ The phased implementation of national chlamydia screening programme in the United Kingdom offers screening for all sexually-active women using NAAT.⁽¹⁵⁾

Several studies have reported a decline in chlamydia prevalence after early screening and proper treatment.⁽¹⁶⁻¹⁸⁾ The best *evidence-to-date* about the effectiveness of screening for chlamydia infection in preventing PID is the randomised controlled trial conducted in Seattle, USA.⁽¹⁹⁾ Two Swedish studies have supported its findings.^(20, 21) Selective screening is more cost-effective than universal screening although the later may be indicated when prevalence of infection is high.^(22, 23)

PATIENTS AND METHODS

The present study aimed to screen the Saudi infertile *sexually-active* women in Jazan city, Saudi Arabia, for possible chlamydia trachomatis infection.

SETTING:

The study was conducted in Jazan General Hospital (JGH) with aid of laboratory facilities at the University of

King Abdulaziz, Jazan city (South-west region of Saudi Arabia).

STUDY PERIOD:

This study covered a period of one year (from July 1st, 2011 to June 30th, 2012).

RECRUITMENT CRITERIA (STUDY GROUP):

The *study group* was included all Saudi married women of primary and secondary infertility, aged between 18 and 40 years-old, who attended the outpatients' gynaecology clinic at JGH for infertility work-up, during the period of study, and agreed to participate (*signed informed consent*).

RANDOMISATION (CONTROL GROUP):

The *control group* was included a randomised 100 Saudi married pregnant women, who attended the outpatients' obstetric clinic for routine antenatal-care, during the period of study, and agreed to participate (*signed informed consent*). Randomisation was done by using the Random Allocation Software[®] (Version 1.0, released on May 2004).

Data were collected by structured meetings with all recruited women where they received "*Information Sheet*" about the present study and signed an "*Informed Consent*".

Relevant medical records were reviewed for any possible present and past medical or surgical diseases. For a precise detection of chlamydia infection, two methods have been used for screening; an indirect method for detection of chlamydia specific IgG and IgA antibodies in the sera (ELIZA), and a direct method for detection of chlamydia organism (McCoy cell culture). All participants' data and screening results were managed confidentially. About 5 milliliters of venous blood was drawn from each participant for measurement of serum chlamydia IgG and IgA antibodies by using peptide-based enzyme-linked immunosorbent (IgG-pELIZA Cat. No. 497/TMB, and IgA-pELIZA Cat. No. 498/TMB Medac[®], Wedel, Germany). Each Serum sample has a numerical code so that clinical condition of tested woman was unknown to the lab technician. All kits used in this study were of the same batch. Each kit contains 96 wells, and all tests were performed before their printed expiry-dates. The manufacturer's instructions for test procedure were followed when performing the assays. The kits, microplates, controls, buffers, diluent, conjugate, TMB-substrate, and stop solution were stored at 4°C and allowed to stand for one hour at room temperature before use. Results were interpreted according to the manufacturer's instructions, issuing a negative, equivocal, or positive result.

The intensity of the colour is proportional to the concentra-

concentration (titer) of the specific antibody in the sample. Cut-off values were calculated according to the manufacturer's instructions. Samples with optical density values located within the grey zone were retested again after 2 weeks in order to determine a titre change. Results remained in the gray zone were considered negative.

The staff-nurse of the outpatients' clinic and two general practitioners were taught how to collect endocervical and urethral specimens. We used a cyto-brush (collect more columnar cells than cotton-tipped swabs). The brush inserted into the cervical os beyond the squamocolumnar junction, 1-2cm deep, rotated and removed without touching the vaginal mucosa. The urethral swab inserted 1cm into the urethra, rotated once prior to removal, and placed in a separate tube of 2SP culture-transport medium. Examined women instructed not to urinate within the previous hour (as urination washing-out the infected columnar cells). All collected samples were transported on wet-ice to the lab of the university within 12 hours of collection. A strict adherence to the standard techniques of collecting specimens has biased the findings towards screening outcomes. As chlamydia trachomatis is an obligatory intracellular pathogen, a specimen that lacks endocervical cells was discarded as it will greatly increase the probability of a false negative result.

Specimens inoculated into coverslip cultures of McCoy treated cells with cycloheximide. The inoculum centrifuged for one hour. We used one-dram shell vials (more sensitive than multi-wells). Inoculated cultures were incubated at 37° C for 3 days, washed, fixed in methanol, stained with Giemsa, and screened by dark-ground microscopy for intracytoplasmic inclusion bodies. Universal precautions were followed when handling such risky specimens and all our lab workers were vaccinated against hepatitis-B virus.

After the screening reports have been collected, all participants were informed of their test-results and discussed with the staff-nurse its implications. Any woman has a positive result was referred to the clinic for treatment with a notification letter for her husband to be tested and treated.

STATISTICAL ANALYSIS:

The collected clinical and laboratory data were stored on Microsoft® Excel spreadsheets for Windows and then analysed by the suitable statistical methods using the StatsDirect® Statistical Software (Version 2.7.9, released on 9th July 2012). The data were double-checked carefully during the process of collection, transcription, and computer entering. No missing data has been encountered. For testing the significance, t-test and Yates-corrected Chi square test with Fisher exact at confidence interval of 95% and significance level of 5% have been used. A finding was considered of statistical significant if *p-value* is < 0.05.

RESULTS

The rate of chlamydia trachomatis infection among infertile Saudi women was higher than its rate among the fertile Saudi women (Table: 1, Figure: 1).

The mean age of infertile Saudi women enrolled in this study as *study group* was 26.4 ± 4.8 years-old. Women aged less than 25 year-old were 276 women with an infection rate of 7.81%, while those aged between 25 to 40 years-old were 364 women with an infection rate of 7.19% (Figure: 2). This was not of statistical significance (CI = 0.97-2.42, OR = 1.53, *p-value* = 0.0581).

Most of infertile Saudi women were having primary infertility (425 women) with an infection rate of 11.72%, while 215 women were having secondary infertility with an infection rate of 3.28% (Table: 2, Figure: 3).

The mean of infertility duration for the *study group* was 3.28 ± 1.73 years. Cases with infertility duration less than 5 years were 498 women with an infection rate of 12.5%, while those with infertility duration longer than 5 years were 142 women with an infection rate of 2.5%. This finding was not of statistical significance (CI = 0.84-2.86, OR = 1.51, *p-value* = 0.1832).

Among infertile Saudi women, a past history of STI was mentioned by 165 women (25.78%), while 70 women (10.94%) were reported a previous pelvic surgery. A history of new husband in the preceding year was mentioned by 47 women (7.34%), while 23 women (3.59%) were reported a past use of intra-uterine contraceptive copper-T device.

Most women of the *study group* were asymptomatic (475 women) with an infection rate of 11.72%, while 165 women were symptomatic with an infection rate of 3.28% (Figure: 4). This finding was not of statistical significance (CI = 0.75-2.28, OR = 1.29, *p-value* = 0.3777).

Inquiry has revealed a complaint of painful micturition in 115 women (17.97%), vaginal discharges in 98 women (15.31%), pelvic pains in 96 women (15.0%), irregular uterine bleeding in 47 women (7.34%), postcoital bleeding in 27 women (4.22%), and urethral discharge in 3 women (0.47%). On pelvic examination the signs were; mucopurulent cervicitis in 224 women (35.0%), cervical friability in 132 women (20.63%), and hypertrophic cervix in 56 women (8.75%).

The rate of detection of chlamydia trachomatis infection among infertile women by using ELIZA test was 9.84% while it was 12.03% by using culture technique (Tables: 3 & 4, Figure: 5). This finding was not of statistical significance (CI = 0.55-1.15, OR = 0.798, *p-value* = 0.2443). At last, a predictive analysis for the screening tests used in this study was done (Table: 5).

DISCUSSION

Chlamydia trachomatis infection is a worldwide distributed sexually-transmitted infection. Prevalence of infection is difficult to be estimated without screening as most of cases are asymptomatic. While a total of 929,462 cases of chlamydia infection were reported in USA to CDCP in year 2004,⁽¹⁴⁾ the actual number of cases is thought to be more than 2.8 million per year.⁽²⁴⁾ According to WHO, the new cases of chlamydia infection have been estimated globally to be 92 million.⁽²⁵⁾ Both health and economic consequences of infection are serious thus; prevention and early detection are essential.

Table 1. Prevalence of chlamydia trachomatis infection among Saudi women (Cases vs Controls).

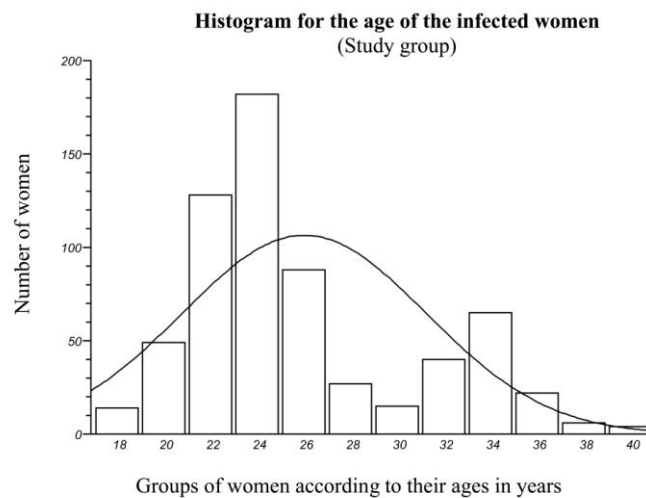
Screening Results	Cases (Infertile Women) n = 640	Controls (Fertile Women) n = 100	Fisher exact 95% CI		p-value	OR
			Lower	Upper		
Infected	96 (15.0%)	4 (4.0%)	1.54	16.21	0.0015	4.24
Not infected	544 (85.0%)	96 (96.0%)				

(n = number of women, CI = Confidence interval, p = significance level, OR = odds ratio).

Figure 1. Prevalence of chlamydia trachomatis infection among Saudi women (Cases versus Controls).



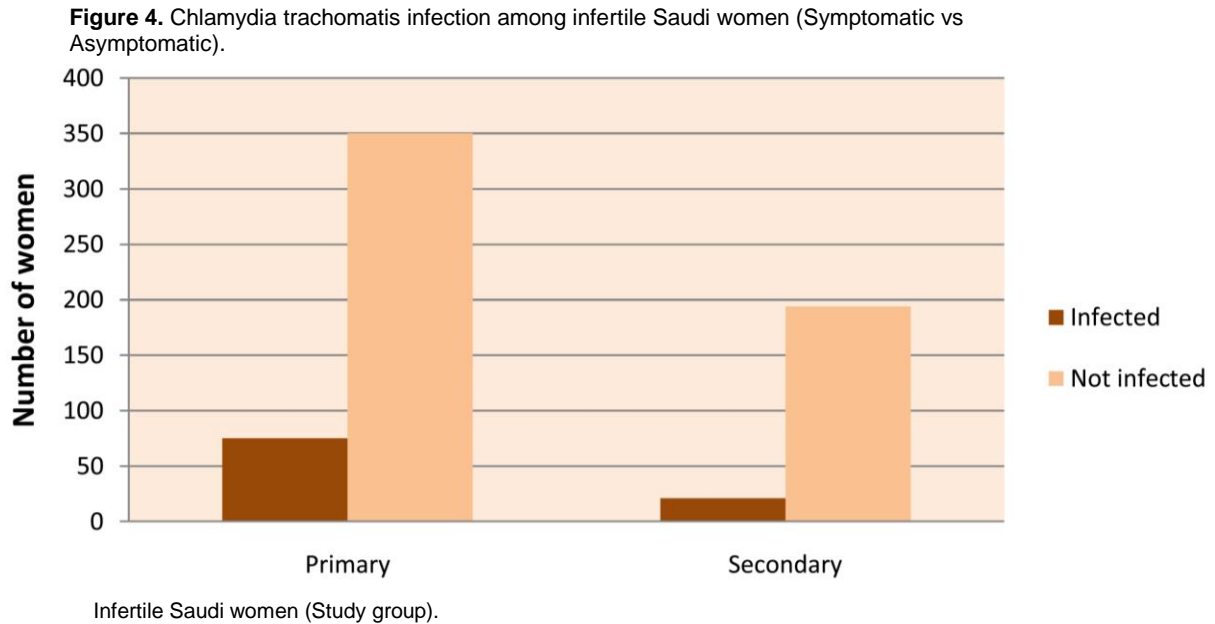
Figure 2. Histogram with distribution curve according to the age of infected infertile women (Positively skewed to the right, i.e. more cases at young age).



The finding of this study is highlighting the need for a chlamydia screening programme as the prevalence of inf-

Table 2. Prevalence of chlamydia trachomatis infection among infertile Saudi women (Primary vs Secondary infertility).

Screening Results	Infertile Cases (Study group)		Fisher exact 95% CI		p-value	OR
	Primary n = 425	Secondary n = 215	Lower	Upper		
Infected	75 (11.72%)	21 (3.28%)	1.16	3.49	0.0096	1.98
Not Infected	350 (54.69%)	194 (30.31%)				

**Table 3.** Interpretation of ELIZA Test results.

IgG	IgA	Test Interpretation	No. of Cases
+	+	Positive chlamydia trachomatis Infection.	27
+	-	Past chlamydia trachomatis Infection.	19
-	+	Early chlamydia trachomatis Infection.	3
-	?	Possible Early chlamydia trachomatis Infection.	0
?	-	Possible Past chlamydia trachomatis Infection.	7
+	?	Past with Possible Early chlamydia trachomatis Infection.	5
?	+	Early with Possible Past chlamydia trachomatis Infection.	2
-	-	Negative chlamydia trachomatis Infection.	577

(IgG: Immunoglobulin-G, IgA: Immunoglobulin-A antibodies, +: Positive, -: Negative, ?: Equivocal).

infection among infertile Saudi women in Jazan city, Saudi Arabia, is high (15.0%). Socio-demographic risk-factors for infection are including; young age, ^(26, 27) urban setting and low-income, ⁽²⁸⁾ having multiple sexual partners, ⁽²⁹⁾ or an infected partner,

⁽³⁰⁾ nulliparity, ⁽³¹⁾ and irregular use of barrier contraceptives or using oral contraceptive pills. ⁽³²⁾ Due to local cultural and social constraints, our study excluded women aged less than 18 year-old. Consequently; direct comparison on chlamydia prevalence could not be made

Table 4. Comparing results of screening tests (ELIZA vs Culture).

Screening Test	Positive	Equivocal	Negative
IgG p-ELIZA	51	9	580
IgA p-ELIZA	32	5	603
McCoy cell culture	77	0	563

Figure 5. Results of screening tests (ELIZA vs McCoy Culture).

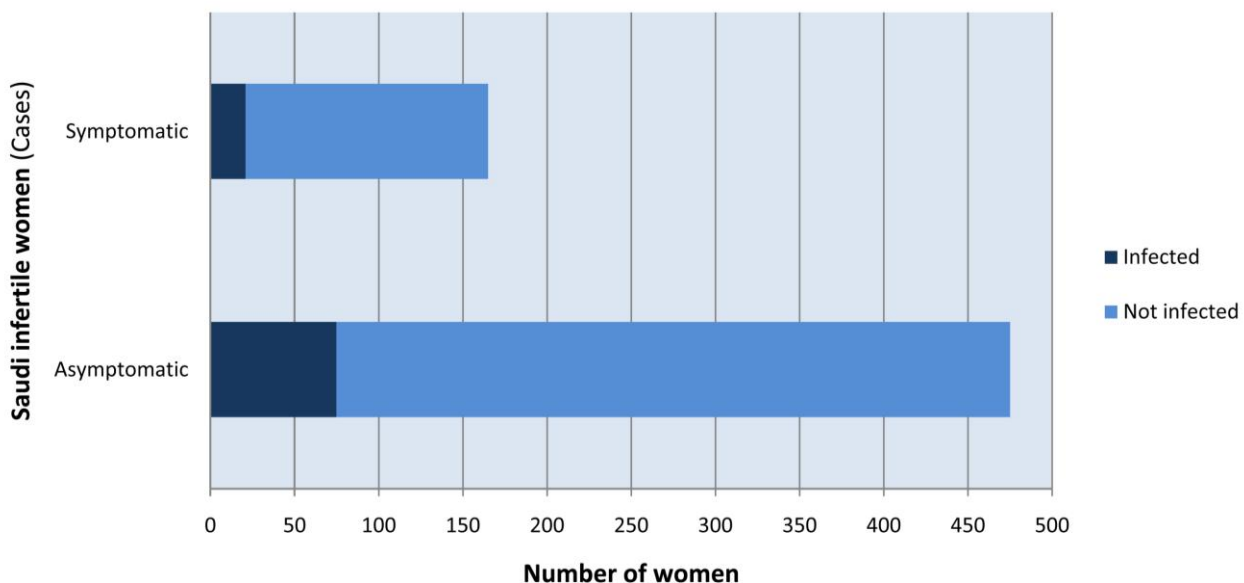


Table 5. Prediction of screening tests (ELIZA vs Culture).

Parameter	ELIZA	McCoy Cell Culture
Sensitivity	65.62%	80.21%
Specificity	100.0%	100.0%
Positive Predictive Value (PPV)	100.0%	100.0%
Negative Predictive Value (NPV)	94.28%	96.63%

precisely with western studies that included younger women (aged 15-19 years-old).⁽¹⁴⁾ Moreover, un-married Saudi women were excluded as the management of any positive cases would create both legal and social problems. Furthermore, routine asking about the numbers of sexual partners is not realistic in an Islamic community.

In our study, the rate of infection was 7.81% for infertile Saudi women aged 18 to less than 25 years-old. This *rate-age* association is largely related to the higher level

of sexual-activity among young women whose squamocolumnar junction of their cervix still presents on ectocervix (*cervical ectopy*) that provides a large target area for infection.⁽³³⁾ That is why, age is used in many countries as a primary determinant for selective chlamydia screening programmes.⁽³⁴⁾

The prevalence of chlamydia infection among asymptomatic healthy-looking Saudi women is 8.5% at Riyadh city,⁽³⁵⁾ while it is 4.0% in our study at Jazan city, Saudi Arabia.

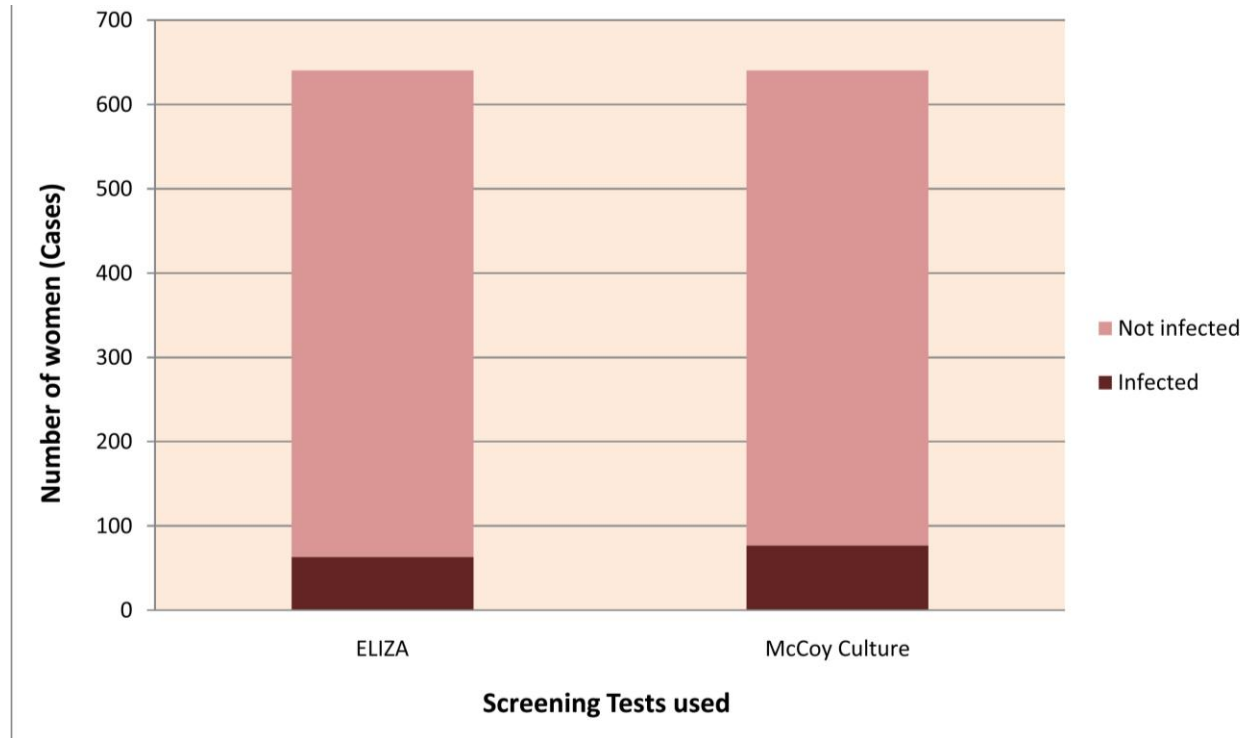


Figure 5. Results of screening tests (ELIZA vs McCoy Culture).

In this study, positive chlamydia was seen in 75 women out of 425 (17.65%) with primary infertility, and in 21 women out of 215 (9.77%) with secondary infertility. In Indian study, positive chlamydia was seen in 20 women out of the 74 (27.03%) with primary infertility, and in 11 women out of the 36 (30.56%) with secondary infertility.⁽³⁶⁾

Although chlamydia infection is not yet a fully reportable infectious disease in Saudi Arabia, sera from patients attended our clinic in Jazan were 9.06% positive for IgG antibodies and 5.0% positive for IgA antibodies. In Makkah city, chlamydia IgG antibodies were detected in 8.7% of pregnant Saudi women,⁽³⁷⁾ while in our study, IgG antibodies were detected in only 4.0%.

This study is considered the first to screen infertile Saudi women at Jazan city for chlamydia trachomatis infection as a possible causative factor for their infertility. Lack of chlamydia screening among Saudi women might result from lack of awareness of high rate of infection among young asymptomatic women and its serious late complications.

As there is a high prevalence of chlamydia infection among infertile Saudi women (*evident in this study*) and a high relationship between chlamydia infection and tubal factor infertility (*evident in literature*), it is mandatory to screen all infertile Saudi women for chlamydia trachomatis at the start of their infertility work-up prior to any invasive procedure.

The limitations of data surveyed in this study are

including; its restriction to the geographic boundaries of Jazan city, to the time frame of one year, and to a specific target risk-group (infertile Saudi women). There are several questions in-need for further researches such as; prevalence of chlamydia trachomatis infection among general population in Saudi Arabia, cost-effectiveness of selective versus universal screening programmes, and the effect of screening programme in reducing the rate of infertility.

CONCLUSION

The prevalence of chlamydia trachomatis infection among infertile Saudi women at Jazan city, Saudi Arabia, is high (15.0%). This finding calls for a national screening programme for chlamydia trachomatis infection among all sexually-active couples in Saudi Arabia.

ELISA test seems to be a good alternative to culture method for screening purpose. It is simple and relatively cheap to the culture technique. It has a high specificity (100%) and a high negative predictive value (94.28%).

DECLARATION

I hereby declare that this work was carried out in accordance with the requirements of the University of Jazan Regulations and Code of Ethics for Research Prog-

rams. It was approved by the Research Review Board. Except where indicated by specific reference in the text, this work is my own work. There was no contribution of any other authors. Any views expressed in the study are those of the author. The work was self-funded. I did not receive any financial funding or support from any person or institution. In addition, I state that I have no competing interests.

REFERENCES

- Mehanna MT, Rizk MA, Eweiss NY, Ramadan M, Zaki SA, Sadek A (1995). Chlamydial serology among patients with tubal factor infertility and ectopic pregnancy in Alexandria, Egypt. *J Sex Transm Dis*; 22(5):317-21.
- Rajkhowa M, Glass MR, Rutherford AJ, Balen AH, Sharma V, Cuckle HS (2000). Trends in the incidence of ectopic pregnancy in England and Wales from 1966 to 1996. *BJOG*;107:369-74.
- 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):1841-47.
- Madani TA (2006). Sexually-transmitted infections in Saudi Arabia. *J. Infectious Dis*;10; 6:3.
- Watson MA, Templeton A, Russell I, Paavonen J, Mardh P-A, Stary A (2002). The accuracy and efficacy of screening tests for chlamydia trachomatis: a systematic review. *J. Med. Microbiol*; 51(11):1021-31.
- Moore DE, Spadoni LR, Foy HM, Wang SP, Daling JR, Kuo CC (1982). Increased frequency of serum antibodies to chlamydia trachomatis in infertility due to distal tubal disease. *Lancet*; 11:574-77.
- Fiddelers AA, Land JA, Voss G, Kessels AG, Severens JL (2005). Cost-effectiveness of chlamydia antibody tests in subfertile women. *Hum Reprod*; 20(2):425-32.
- Chernesky M, Luinstra K, Sellors J, Schachter J, Moncada J, Caul O (1998). Can serology diagnose upper genital tract chlamydia trachomatis infection? Studies on women with pelvic pain, with or without chlamydial plasmid DNA in endometrial biopsy tissue. *Sex Transm Dis*; 25:14-19.
- Grossman JH, Rivlin ME, Morrison JC (1991). Diagnosis of chlamydial infection in pregnant women using the Test-pack Chlamydia diagnostic kit. *J Obstet Gynecol*; 77:801-03.
- Ferris DG, Martin WH (1992). A comparison of three rapid chlamydial tests in pregnant and non-pregnant women. *J Fam Pract*; 34:593-97.
- Blanding J, Hirsch L, Stranton N, Wright T, Aarnaes S, de la Maza L (1993). Comparison of the Clearview Chlamydia, the PACE 2 assay, and culture for detection of chlamydia trachomatis from cervical specimens in a low-prevalence population. *J. Clin. Microbiol*;31:1622-25.
- Schachter J, McCormack WM, Chernesky MA, Martin DH, Van Der Pol B, Rice, PA (2003). Vaginal swabs are appropriate specimens for diagnosis of genital tract infection with chlamydia trachomatis. *J Clin Microbiol*; 41(8):3784-9.
- Garland SM, Gertig DM, McInnes JA (1993). Genital chlamydia trachomatis infection in Australia. *Med J Australia*;159:90-6.
- Centers for Disease Control and Prevention (CDCP). Sexually transmitted disease surveillance 2004 supplement, Chlamydia Prevalence Monitoring Project, 2005. Atlanta, US: Department of Health and Human Services.
- La Montagne DS, Fenton KA, Randall S, Anderson S, Carter P (2004). On behalf of the National Chlamydia Screening Steering Group. Establishing the National Chlamydia Screening Programme in England: results from the first full year of screening. *J Sex Transm Infect*; 80(8):335-41.
- Addiss DG, Vaughn ML, Ludka D, Pfister J, Davis JP (1993). Decreased prevalence of chlamydia trachomatis infection associated with a selective screening program in family planning clinics in Wisconsin. *Sex Transm Dis*;20:28-35.
- Herrmann B, Egger M (1995). Genital chlamydia trachomatis infections in Uppsala county, Sweden, 1985-1993: declining rates for how-much longer? *J Sex Transm Dis*; 22:253-60.
- Katz BP, Blythe MJ, Van der Pol B, Jones RB (1996). Declining prevalence of chlamydial infection among adolescent girls. *J Sex Transm Dis*; 23:226-29.
- Scholes D, Stergachis A, Heidrich FE, Andrilla H, Holmes KK, Stamm WE (1996). Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection. *N Engl J Med*; 334:1362-66.
- Kamwendo F, Forslin L, Bodin L, Danielsson D (1996). Decreasing incidences of gonorrhoea and chlamydia associated acute pelvic inflammatory disease. A 25-year study from an urban area of central Sweden. *J Sex Transm Dis*; 23:384-91.
- Egger M, Low N, Smith GD, Lindblom B, Herrmann B (1998). Screening for chlamydial infections and the risk of ectopic pregnancy in a county in Sweden: ecological analysis. *BMJ*; 316:1776-80.
- Sellors JW, Pickard L, Gafni A, Goldsmith CH, Jang D, Mahony, JB (1992). Effectiveness and efficiency of selective vs universal screening for chlamydial infection in sexually active young women. *Arch Intern Med*; 152:1837-44.
- Marrazzo JM, Celum CL, Hillis SD, Fine D, DeLisle S, Handsfield HH (1997). Performance and cost-effectiveness of selective screening criteria for chlamydia trachomatis infection in women. Implications for a national chlamydia control strategy. *J Sex Transm Dis*; 24:131-41.
- Weinstock H, Berman S, Cates W (2004). Sexually-transmitted diseases among American youth: incidence and prevalence estimates, 2000. *Perspect Sex Reprod*

Health; 36(1):6-10.

World Health Organization. Global Prevalence and Incidence of selected curable Sexually Transmitted Infections: Overviews and estimates, 2001.

Macmillan S, McKenzie H, Flett G, Templeton A. Which women should be tested for chlamydia trachomatis?. *BJOG*107(9):1088-93.

Tobin JM. Chlamydia screening in primary care: is it useful, affordable and universal? *J Curr Opin Infect Dis* 2002;15:31-36.

Gunn RA, Hillis SD, Shirey P, Waterman SH (1993). Greenspan JR. Chlamydia trachomatis infection among Hispanic women in the California-Mexico border area: establishing screening criteria in a primary care setting. *J Sex Transm Dis* 1995;22:329-34.

Mosure DJ, Berman S, Kleinbaum D, Halloran ME (1996). Predictors of chlamydia trachomatis infection among female adolescents: a longitudinal analysis. *Am J Epidemiol*. 144:997-1003.

Rietmeijer CA, Van Bemmelen R, Judson FN, Douglas JM Jr (2002). Incidence and repeat infection rates of chlamydia trachomatis among male and female patients in an STD clinic: implications for screening and re-screening. *J Sex Transm Dis*. 29(2):65-72.

Cook RL, St. George K, Lassak M, Tran N, Anhalt JP, Rinaldo CR (1999). Screening for chlamydia trachomatis

infection in college women with a polymerase chain reaction assay. *J Clin Infect Dis*. 28:1002-07.

Cottingham J, Hunter, D. Chlamydia trachomatis and oral contraceptive use: a quantitative review. *J Genito-urinary Med* 1992;68:209-16.

Centers for Disease Control and Prevention (CDCP). Sexually transmitted disease surveillance 2003 supplement, Chlamydia Prevalence Monitoring Project, 2004. Atlanta, US: Department of Health and Human Services.

Miller WC, Hoffman IF, Owen-O'Dowd J, McPherson JT, Privette A, Schmitz JL (2000). Selective screening for chlamydial infection: which criteria to use?. *Am J. Prev Med*.18(2):115-22.

Bakir TM, Hossain A, De-Silva S, Siddiqui A, Sengupta BS, El-Sheikh MM (1989). Enzyme immunoassay diagnosis of chlamydia trachomatis infections in diverse patient groups. *J. Hyg Epidemiol Microbial Immunol*. 33(2):189-97.

Malik A, Jain S, Hakim S, Shukla I, Rizvi M (2006). Chlamydia trachomatis infection and female infertility. *Indian J Med Res* 2006;123(6):770-75.

Ghazi HO, Daghestani MH, Mohamed MF (2006). Seropositivity of chlamydia trachomatis among Saudi pregnant women in Makkah. *J Family & Comm Med*. 13(2): 61-4.