Muntaha M. H. AL-Alouci et al /J. Pharm. Sci. & Res. Vol. 11(3), 2019, 1131-1135

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Assessment of Chlamydia trachomatis infection in symptomatic women by ELISA and evaluate the levels of CRP, C3, C4 and IgA in patients sera

Muntaha M. H. AL-Alouci* Noor N. R. AL-Hayani Tamadher M. H. AL-Alouci *****

**College of Medicine/ University of Anbar/Iraq,*
***College of Medicine/ University of Anbar/Iraq,*
****AL-Ramadi Hospital for gynecology and pediatrics/Iraq.*

Abstract:

Background: *Chlamydia trachomatis* is the causativeof a widespread sexually transmitted infection. Untreated *C. trachomatis* infection has been linked to serious long-term squeal, such as ectopic pregnancy and tubal infertility. *C. trachomatis* diagnosis is not always easily accomplished. Innate immunity is of key importance in primary recognition of invading pathogens, acquired immune response; both humoral and cell-mediated in this infection is detectable from 1-2 weeks after the primary infection.

Objective: This study has been undertaken to detect specific IgM and IgG antibodies against *C. trachomatis* in blood samples in patients with genital tract infection quantitatively by ELISA. Also toevaluate total IgA, C3,C4 and CRPas immunoparameters in infected patients.

Patients and Methodology: Blood samples were taken from 80 women and the sera were used to determine the level of specific IgG and IgM antibodies against *C. trachomatis*. Quantitative determination of anti *C. trachomatis* IgG and IgM antibodies were performed by ELISA using (NovaLisa *C. trachomatis* IgM &IgG). The level of CRP was determined by using a CRP kit (Omega, UK). The quantifications of IgA and complement components (C3 and C4) were performed by radial immunodiffusion (RID).

Results: Out of 80 enrolled in this study, IgM was found in 3(3.75%) and IgG was found in 5 (6.25%) of them as detected by the technique of Enzyme Linked Immunosorbent essay. The two –sample t-test was used in order to compare means of IgM, IgG with IgA and CRP. The t- test revealed that there is no significant difference between CRP for IgM and IgG, no significant differences between IgA with IgM but there is significant correlation between IgA and IgG and IgA with C3, C4 correlated with C3 only.

Conclusions: Means of IgM and IgG were compared with respect to CRP, IgA, C3 and C4 as immunoparameters in age group ranged from (16-46 years). In both comparisons found to have no significant differences between CRP with IgG and CRP with IgM. IgA appear to be correlated with IgG and C3 and C4 correlated with C3 only.

Keywords: *Chlamydia trachomatis*, Immunoglobulins, C3,C4, ELISA.

INTRODUCTION:

*Chlamydia trachomatis*cause a widespread sexually transmitted infection. It has been assessed that 20% of women with lower genital tract chlamydial infection will progress pelvic inflammatory disease (PID), 4% develop chronic pelvic pain, 3% infertility, and 2% adverse pregnancy outcome.

The most common bacterial sexually transmitted disease in Europe is caused by Chlamydia trachomatis, and a high prevalence of infection has been documented among healthy women[(1).] *Chlamydia trachomatis* infection may cause urethritis, cervicitis and pelvic inflammatory disease in women. Since more than 50 % of *C. trachomatis* infections are asymptomatic, they may stay undetected, and thus untreated, for prolonged periods of time. Untreated *C. trachomatis* infection has been associated to severe long-term squealed, such as ectopic pregnancy and tubal infertility[(2, 3, 4)]

To get valid data it is essential to have the right test specimen, but in C. trachomatis diagnosis, this is not always easily realized. The infection might be limited in the endometrium, fallopian tubes, ovaries or the prostatic tissue, Which are not easily reachable, and it requires invasive processes to get tissue samples from these positions. A first void urine sample or a cervical or urethral swabis the common routine diagnosis involved. Vaginal swabs have been introduced with good accuracy for detection of lower genital tract infections. On the other hand, C. trachomatis antigen or DNA has been found in endometrium, fallopian tubes, ovaries, semen or prostatic tissue without being able to detect any bacteria in urine specimens or cervical secretions. Although Chlamydia are bacteria, they are obligate intracellular pathogens. Methods for isolation in culture are similar to those used in the virology laboratory. Inclusion bodies of *C. trachomatis* contain glycogen which may be stained and used for diagnosis by staining with Giemsa stain or Geminza methods. Fluorsceinated monoclonal antibodies are somewhat greater sensitivity because specific monoclonal antibodies directed against outer-membrane proteins and lipopolysaccharide [(5,6, 7, 8)]

Chlamydia serology, utilizing a blood sample, might in some instances be an alternative diagnostic method with an easily accessible test specimen giving information on the immune reaction to a Chlamydial infection at any site of the body. The enzyme immunoassay (EIA) is a commonly used front line assay for the diagnosis of CT infection. Molecular methods are more sensitive than other methods, but they are also more expensive.The ELISA was based on a synthetic peptide from the immunodominant region of the

major outer membrane protein.(9, 10). The ELISA kit used in this study was very specific for identifying*C. trachomatis* without any cross activity with other species of Chlamydia

PATIENTS AND METHODOLOGY:

Patients:

Eighty married females were selected from the department of the gynecology and obstetrics of the AL-Ramadi teaching hospital for obstetric and pediatrics depending on signs and symptoms by the physicians from November 2012 to April 2014, thirty healthy people with the same socioeconomic standard and age groups were evaluated as control group. All cases and controls were subjected to full history including the period of onset of the current illness, fever, cough, age and pregnancy.

Serological tests:

Blood samples were taken from 80 women and the sera were used to determine the level of IgM and IgG antibodies against *C. trachomatis*.Quantitative determination of anti *C. trachomatis* IgG and IgM antibodies were performed by ELISA using (NovaLisa *C. trachomatis* IgM &IgG). According to the manufacture instructions ,samples with antibody index below 9 was considered as not having anti *C. trachomatis* antibodies , samples with Ab index above 11 were considered as positive and having specific. Samples with antibody index of (9-11) were considered equivocal.The level of CRP was determined by using a CRP kit (Omega, UK). The sensitivity of the CRP test was 6 mg/L. Both tests were performed according to the manufacturers' instructions. The quantifications of IgA and complement components were performed by radial immunodiffusion (RID). RID assays were based on antigen-antibody precipitation reaction. Antigens were added to wells coated in an agarose matrix which contains an optimal concentration of monospecific antiserum. The incubation period lasted 48-72 hrs. the specific complement protein diffused into the agar, forming a precipitation ring in its interaction with antibodies. The diameter of the rings reflects the concentration of complement proteins present in the tested sera. The precise amount was determined by comparing the diameter of the precipitin ring formed with standard solutions of complement protein.

Statistical analysis:

Statistical package for social sciences (SPSS) version 17 was used for the statistical analysis. P value of less than 0.05 was considered significant.

Mean ± standard deviation, standard error was used to describe quantitative data. Number and percentage was used to describe categorical data.

Student t-test was used to compare +ve and –ve cases of CRP for IgG and IgM, Chi-square test was used to find the relation of categorical data. Correlation test was used to test the correlation of quantitative data.

RESULTS:

The study revealed that the age of patients was ranged from (16- 46 years), mean age was 27.6± 7.68SD. Out of 80 enrolled cases in this study, specific IgM for *Chlamydia trachomatis* found in 3(3.75 %) and specific IgG for *Chlamydia trachomatis* found in 5(6.25 %) of them as detected by the technique of Enzyme Linked Immunosorbent Assay as represented in Table-1**.**

Table 1: Detection of *Chlamydia trachomatis* infection by ELISA

No. of patients	Specific IgM		Specific IgG	
	No	%	No	%
80	3	3.75	5	6.25

The positivity and negativity of CRP was measured also, level of specific IgM and IgG were measured also, according to the statistical analysis there is no significant difference between CRP and both IgG and IgM as revealed in **Table -2.**

Table 2: Comparisons of Means CRPwith IgG and IgM

	CRP	N	Mean	Std. Deviation	Std. Error Mean	P value
IgG	Positive	46	6.8913	4.08998	.60303	0.961 NS
	Negative	34	6.8441	4.43493	.76058	
IgM	Positive	46	4.4196	3.06715	.45223	0.518 NS
	Negative	34	3.9794	2.89260	.49608	

According to the result of study, there is no significant relation between specific IgG and IgA (p= 0.397), the majority of < 10 IgG cases with normal IgA level (90-450) and no significant relation between specific IgM and IgA (p= 0.190), the majority of < 10 IgM cases with normal IgA level (90-450) as represented in **Table 3, Figure-1 ,Table-4, Figure -2.**

Table 3: Comparisons of Means IgA with IgG

			IgA		Total
			90-450	>450	
IgG	<10	Count	58	8	66
		%	87.9%	12.1%	100.0%
	>=10	Count	11	3	14
		%	78.6%	21.4%	100.0%
Total		Count	69	11	80
		%	86.3%	13.8%	100.0%

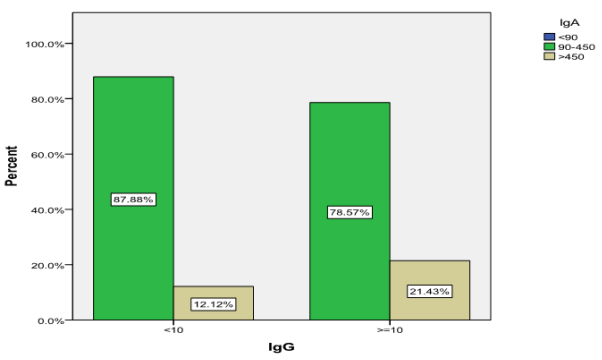

Figure-1: Comparisons of Means IgA with IgG

Table 4: Comparisons of Means IgA with IgM

			IgA		Total
			90-450	>450	
IgM	<10	Count	65	9	74
		%	87.8%	12.2%	100.0%
	>=10	Count	4	2	6
		%	66.7%	33.3%	100.0%
Total		Count	69	11	80
		%	86.3%	13.8%	100.0%

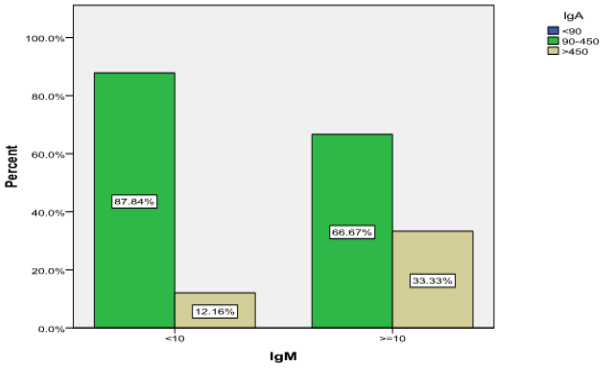

Figure-2: Comparisons of Means IgA with IgM

Regarding IgA, IgM and IgG results, there is a significant correlation between IgA and IgG (p= 0.007, r=0.300), while there is no significant correlation with IgM (p=0.228, r=0.136). The concentration reading of IgA and complement 3 were revealed in **Table 5** and **Figure3**. The relationship between IgA and C3 were investigated, there is a significant relation between C3 and IgA (p= 0.041). The lower C3 values are associated with higher IgA values are more than the higher C3 values that are associated with lower IgA values.

Table-5: The relation of IgA with C3

			IgA		Total
			90-450	>450	
C3 C4	<91	Count	19	7	26
		%	73.1%	26.9%	100.0%
	91-156	Count	38	2	40
		%	95.0%	5.0%	100.0%
	>156	Count	12	2	14
		%	85.7%	14.3%	100.0%
Total		Count	69	11	80
		%	86.3%	13.8%	100.0%

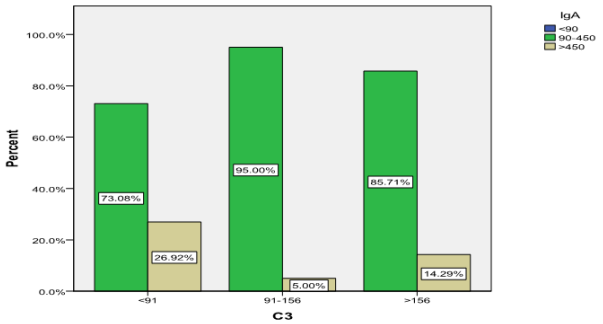

Figure-3: The relation of IgA with C3

Regarding to C3 and IgG results, C3 is correlated with IgG (p=0.008 r=0.296). It is clear that C4 is correlated with C3 only (p=0.042 r=0.228).overlap

DISCUSSION:

Chlamydia trachomatis is a common sexually transmitted infection with significant influence on public health. Therefore, effective epidemiological control as well as a correct and sensitive diagnostic method for *C. trachomatis* is required, so the presented study aim to determine the prevalence of *Chlamydia trachomatis* and their association with some immunological parameters**.**

Local and mucosal IgG antibodies can persist in the lower genital tract mucosa for a long time (similar to IgG in serum)andas markers of a previous infection, *C. trachomatis* IgG antibodies may be predictor of infection and antibiotic treatment may cure a hidden residual infection since a correlation between *C. trachomatis* antibodies and findings of antigens in samples[(11),]

In the present study IgM and IgG reported low level as immunological markers, this may be due to many reasons like that the serological tests depends on the site of infection, duration of disease and because adherence of moral principles and code of ethics in the region of study

A similar study in Iran showed that, (6%) infertile and (1.6%) fertile women were positive for IgM (p=0.21). Also, PCR was positive for C. trachomatis infection in infertile (5%) and in fertile women (1.6%) (p=0.35). They did not find any seropositive immunoglobulin G in both groups.(12)

Other study done amongSaudi pregnant women in Makkah,Chlamydia trachomatis IgG antibodies were detected in 8.7% and IgM antibodies were found in 1.5% of different age groups.(13)

Antibodies for Chlamydia trachomatis were found in 66 (46%) of 145 male patients and 72 (35%) of 200 female patients attending a genitourinary clinic and gynaecological clinic respectively and in two (2%) of 100 men and in none of 100 women without genital diseases. These results suggest that the prevalence of chlamydial infection in Saudi Arabia among both men and women is high.(14)

Several studies from different countries report different level of C. trachomatis; United Arab Emirates (2.6 %) (15), Jordan (3.9 %) (16), Qatar (5.3 %) (17).

In the presented study there were no differences in mean age (27.6± 7.68SD) of seropositive and seronegative patients for *C. trachomatis*.

The study didn't reveal any differences in mean level of CRPin all age groups of patients. Bendnareks S. A**. (**18) showed the same results in chronically hemodialyzed patients. previous studies revealed that the test combination *C. trachomatis* IgG/CRP might be a better screening method for tubal pathology as compared to the current method *C. trachomatis* IgG only, we foundvery low percentage of positive IgG,IgM specific for C. trachomatis, so the prevalence of *C. trachomatis* as STD in Ramadi city was very low this may due to the usual habit of individuals in the region.

Black (19) reported the immunoassay kit used to detected serum IgG, IgM antibodies against C. trachomatis. However he reported that the presence of immunoglobulin M antibodies was an unreliable marker of acute infection in adolescent and adults.

While IgA antibodies may disappear in the course of time, provided that the infection is cleared. IgA antibodies in serum are assumed to reflect chronic inflammation and have been shown to be useful in the serodiagnosis of tubal factor infertility caused by C. trachomatis. Regarding IgA, IgM and IgG results, there is a significant correlation between IgA and IgG , while there is no significant correlation with IgM. Anti-Chlamydia IgA in serum has also been linked to another complication of Chlamydia. Hence, IgA most likely indicates an active, on-going, persistent infection, rather than a past, cleared infection, which makes it a potential candidate for the biomarker we are looking for **(20)**

This study revealed sero- positive of IgA in chlamydia trachomatisinfected patients was higher, this finding may agree with the report of researchers **(21, 22)**

Chlamydia trachomatis IgA and CRP are serological markers of persistent infection recorded high level in this study. Den Hartog JE**(23)** recorded the same result that both IgA and CRP were significantly more prevalent in women with tubal pathology as compared to those without tubal pathology. The chronic status in the course of a *C. trachomatis* infection is one of the most important aspects of this infection. It is associated with the persistence of the bacteria in the host cells that increases the risk of tubal factor subfertility. IgA antibodies are assumed to reflect chronic inflammation. A level of C-reactive protein (CRP) >10 mg/dls usually occurs in acute infections, and can be detected using common tests for CRP. A CRP level <1 mg/dl indicates the absence of inflammation or infection. Because CRP is a general serological marker of acute inflammation, another object of this study is to detect the serological markers of acute and chronic infections in patients.

The enzyme immunoassay (EIA) is a commonly used front line assay for the diagnosis of CT infection. Molecular methods are more sensitive than other methods, but they are also more expensive **(24)**.

Alteration in the concentration of complement components occurs in a variety of diseases and stage of disease. Strictly intracellular parasitic bacteria (Chlamydia, Rickettsia) do not survive in the extracellular environment of the host, they infect endothelial and epithelial cells and monocytes.(**25)** Cell-mediated immunity and especially participation of type 1Th cells are crucial for eradication or limitation of infection.

C. trachomatis is ubiquitous there is a high prevalence of antibodies in sexually active population. Immunoglobulin M production is believed to be transient and rises in IgM titers are found infrequently .

REFERENCES:

1. Wilson J. S., Honey E., Templeton A., et al. (2002) A systemic review of prevalence of *Chlamydia trachomatis* among European women. *Hum Reprod Update*. 8, 385-394.
2. Gonzales G. F., Munoz G., Sanchez R., et al. (2004). Update on the impact of *Chlamydia trachomatis* infection on male fertility. *Andrologia* 36, 1-23.
3. Hu D., Hook E. W. and Goldie S. J. (2004). Screening for *Chlamydia trachomatis* in women 15-29 years of age: a cost-effectiveness analysis. *Ann Intern Med.* 141, 501-513.
4. Schneede P., Tenke P. and Hofstetter A. G. (2003). Sexually transmitted diseases (STDs)- a synoptic overview for urologists. *Eur Urol.* 44, 1-7.
5. Berit A., Jens K. M., Irene V. V., et al. (2011). Impact of intensified testing for urogenital *Chlamydia trachomatis* infections: A randomized study with 9-year. Sexually Transmitted infections. 87(2):156-161.
6. Scholes D., Stergachis A., Heidrich F. E., et al. (1996). Prevention of pelciv inflammatory disease by screening for cervical chlamydia infection. *N Engk J Med.* 334: 1362-01366.
7. Andersen B., Olesen F., Moller J. K., et al. (2002). Population-Based Strategies for Outreach Screening of Urogenital *Chlamydia trachomatis* infections: A Randomised Controlled Trial. *J Infect Dis.* 185: 252-258.
8. Low N., Bender N., Nartey L., et al. (2009). Effectiveness of chlamydia screening: systematic review. *Int J Epidemiol.* 38: 435-448.
9. Bax C. J., Mutsaers J. E., Jansen C. L., et al. (2003). Comparsion of serological assays for detection of *Chlamydia trachomatis* antibodies in different groups of obstetrical and gynecological patients. *J Clin Lab Immunol.* 10(1): 174-176.
10. Das S. and Allan S. (2006). Higher vaginal pH is associated with *Neisseria gonorrhoeae and Chlamydia trachomatis* infection in a predominantly white population. *Sex Transm Dis*. 33(8): 527-528.
11. Land J. A. and Den Hartog J. E. (2006). Chlamydia antibody testing in subfertile women. Drugs Today (Bare). 42: 35-42.
12. Joolayi F1, Navidifar T1, Mohammad Jaafari R2, Amin M. Comparison of Chlamydia trachomatis infection among infertile and fertile women in Ahvaz, Iran: A case-control study. Int J Reprod Biomed (Yazd). 2017 Nov;15(11):713-718.
13. Hani O. Ghazi, PhD, Mazin H. Daghestani, MD, and Mohamed F. Mohamed, MSc.SEROPOSITIVITY OF CHLAMYDIA TRACHOMATIS AMONG SAUDI PREGNANT WOMEN IN MAKKAH.J Family Community Med. 2006 May-Aug; 13(2): 61–64.
14. Massoud M1, Noweir A, Salah M, Saleh WA Chlamydial infection in Riyadh, Saudi Arabia.B- J Egypt Public Health Assoc. 1991;66(3-4):411-9.
15. Ghazal-Aswad S, Badrinath P, Osman N, Abdul-Khaliq S, Mc Ilvenny S, Sidky I. Prevalence of Chlamydia trachomatis infection among women in a Middle Eastern community. BMC Womens Health. 2004;4(1):3. doi: 10.1186/1472-6874-4-3.
16. Al-Ramahi M, Mahafzah A, Saleh S, Fram K. Prevalence of Chlamydia trachomatis infection in infertile women at a university hospital in Jordan. East Mediterr Health J. 2008;14(5):1148–54. [PubMed]
17. Al-Thani A, Abdul-Rahim H, Alabsi E, Bsaisu HN, Haddad P, Mumtaz GR, et al. Prevalence of Chlamydia trachomatis infection in

the general population of women in Qatar. SexTransm Infect. 2013;89 Suppl 3:iii57–60.
18. Bednarek S. A., Majdan D. M. and Ksiazek A. (2005). Chlamydia trachomatis infection in chronically hemodialyzed patients. RocZ. Akad Med. Bialymst. 50: 307-310.
19. Black C. M. (1997). Current methods of laboratory diagnosis of Chlamydia trachomatis infections. Clin Microbiol Rev. 10(1): 160-184.
20. Idahl A., Boman J., Kumlin U., et al. (2003). Demonstration of *Chlamydia trachomatis* IgG antibodies in the male partner of the infertile couple is correlated with a reduced likelihood of achieving pregnancy. Hum. Reprod. 19(5): 1121-1126.
21. Henry J. C., devries, Vitaly S., et al. (2009). Chlamydia. Clinical Infections Disease. 48(5): 53-56.
22. Dele-Ochiep E, Ifeany M. O., Ngwu A. M., et al. (2016). Serum levels of Immunoglobuline A, G and M in Chlamydia trachomatis infection among primary and secondary infertility patients. British Microbiology Research Journal. 12(5): 1-6.
23. Hartog J. E., Morre S. A., Land S. A., et al. (2009). Sexually Transmitted Infections and Sexually Transmitted Diseases. Infect Immun. 77, 867-876 .
24. Jan E. A. and Robinide Vies. (2006). Systemic screening for Chlamydia trachomatis: Estimating Cost-Effecting Veness Using Dynamic Modeling and Dutch Data. ELSEVIER. Value in Health. 9(1):1-11.
25. William R. McCabe, M.D (1973). Serum Complement Levels in Bacteremia Due to Gram-Negative Organisms. N Engl J Med; 288:21-23