Using pgp2, pgp3 and chxR genes as Agenetic Markers for Detecting the Infection with Chlamydia trachomatis in Women with Ectopic Pregnancy Vaccinated Against SARS-Covid -19

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Abstract

Chlamydial infection is one of the common causative agents of ectopic pregnancy in the cervix of symptomatic /asymptomatic woman . The current study includes 340 endocervical swab samples collected from women suffering from signs or symptoms of ectopic pregnancy, the presence of C. trachomatis infection, abdominal discomfort, and vaginal bleeding by the gynecologist and had risk factors for this infection. A total of (340) women aged between (15->44) years were diagnosed by the consultant in Mosul Hospital ,Mosul ,Nineveh ,Iraq and Al-Batool Hospital ,Mosul ,Nineveh ,Iraq during the period(from Augest 2022 to Fabruary 2023).

The aim of this study is to Chlamydia trachomatis in patients with (EP) by using Giemsa stain ,immunoprecipitation and molecular detection techniques for pgp2,pgp3,and chxR genes diagnostic of C. trachomatis infection . from each women 5 ml of venous blood was collected from each sample to detect the Immunoglobulin (IgG) for women vaccinated with SARS-Covid -19 by using rapid immunoprecipitation.The result showed that C.trachomatis was presented in 27/340 (7.9%) diagnosed by Giemsa stain ,21/340 (6.1%) diagnosed by immunoprecipitation, and 11/340 (3.2%) diagnosed by conventional PCR.Out of 6/182 (3.3%) of vaccinated women who were infected with C. trachomatis , women had (EP) 1/6 was IgG positive and for non vcccinated women, Chlamydia infection was detected in 21/158(13.3%) women.

Pgp2,pgp3 and chxR genes used as genetic markers via conventional PCR by using primer designed in this study all of them gave approximate results 11/340 (3.2%) (all the positive are from ectopic pregnancy women).

Also, the results showed that the isolation rate in age group (25-34) years was the more dominate than other age groups.

Keywords: *Chlamydia trachomatis, Ectopic pregnancy, pgp3, pgp3 and ChxR.*

1- INTRODUCTION

Chlamydia are Gram-negative obligate intracellular parasites.have unique reproduction life cycle. It begins with the attachment of an elementary body (EB) to the host cell surface. EBs are 0.2 to 0.6 mm in diameter, contain dense nuclear material and a rigid cell wall, and are Infectious agent [1] .

Host cells become infected with EBs by endocytosing them an EB is held in an endosome that evades fusion with lysosomes. Then differentiates into a reticulate body (RB) and undergoes binary fission, which continues until the host cell dies. Reticulate bodies are 0.5 to 1.5 mm in diameter and have less dense nuclear material and more ribosomes than EBs; their walls are also more flexible. After 20 to 25 hours, RBs differentiating into infectious EBs. The host cell lyses and releases EBs 48 to 72 hours after Infection[31]. C. trachomatis serovars are human pathogens causing mostly ocular and genital infections. These infections affect millions of people worldwide and if left untreated can lead to blindness or sterility[32]. C. trachomatis strains have classified to three biovars, which can be further divided into 15 main serovars, based on antigenic variation of the major outer membrane protein (MOMP): the trachoma biovar (serovars A-C); the genital biovar (serovars D-K); and the lymphogranuloma venereum (LGV) biovar (serovars L1- L3). Most C. trachomatis infections are caused by genital strains [2].

Chlamydia trachomatis is one of the sexually transmitted diseases. Its infection mostly is asymptomatic, without antibiotic intervention, long-term C. trachomatis infection of the female genital tract may result in chronic inflammatory pathologies ,may cases behind immunopathological tissue damage pelvic inflammatory diseases,(EP), and infertility [3] C. trachomatis isolates share a 7.5-kb virulence-associated plasmid [4]

Carrying eight open reading frames (ORF) encoding proteins designated plasmid gene proteins 1 to 8 (Pgp1 to Pgp8). Pgp1, Pgp2, Pgp6, and Pgp8 are important for plasmid maintenance .Pgp4 is a main positive regulator of plasmid-encoded Pgp3 and the chromosomal genes, including GlgA, CT049-CT050, and CT142-CT144 , whereas Pgp5 is a negative regulator of Pgp4-regulated genes. Pgp7 is a homologue of Pgp8 and shows homology to integrases. The plasmid encodes two small antisense RNAs implicated in plasmid maintenance [5].

Antimicrobial peptides (AMPs) are the first line of innate defense against invading microorganisms of skin and mucosal surfaces. The female reproductive tract expresses multiple AMPs, including defensins, cathelicidin, S100 proteins, C-type lectins, and iron metabolism proteins [8]. Defensin and cathelicidin (LL-37) are known to kill chlamydial organisms by lysing infectious elementary bodies EBs [7]. Bacterial pathogens have different strategies to counteract AMPs to promote colonization and infection [8]. Pgp3 is secreted from the inclusion bodies into the host cytosol [10] and neutralizes the antichlamydial activity of cathelicidin LL-37 in in vitro cell culture assays [9]. Chlamydiae are characterized by a unique biphasic developmental cycle that alternates between an extracellular, metabolically inactive, infectious elementary body (EB) and an intracellular, metabolically active, non-infectious reticulate body RB (6). The regulation of the developmental cycle is one of many aspects that is not completely understood. ChxR (CT630) has been proposed to function in the chlamydial developmental cycle as a RB-to-EB conversion stage-specific transcriptional activator (11). The highly conserved nature of ChxR, its uniform presence in all chlamydial species[11].

The recognized gold standard for C. trachomatis detection for a long time was culture. McCoy cell lines require rather complicated and time-consuming cultivation procedures, as well as skilled laboratory technicians for accurate microscopy and follow-up staining [34]. Sensitivity of culture methodology is much less compared to nucleic acid amplification testing NAAT [12]

2-MATERIALS AND METHODS

2.1 Participants

Clinical samples were collected from(340) Women aged(15-<44) admitted to the out-patient clinics of Gynecology and Obstetrics, in Mosul Hospital ,Mosul ,Nineveh ,Iraq and Al-Batool Hospital ,Mosul ,Nineveh ,Iraq during the period(from Augest 2022 to Fabruary 2023).

These females were diagnosed by the gynecologist as having (EP), according to the characteristic criteria of national guidelines for (EP) disease [16] and according to the signs and symptoms, abdominal and pelvic ultrasound, in addition to having risk factors.

Detection of C. trachomatis Infection

Three plastic swabs were used for each patient; as the specimens were collected by inserting swab about 1 cm into the endocervical canal and were rubbed by rotating it against the wall of endocervical canal vigorously or scraping to get more cells from the endocervix. Then, swabs were removed carefully to avoid any contact with vaginal secretions[33].The swabs were distributed as follows:

1- The first was placed in a dry tube for stainig to detect inclusion bodies .

2- The second was used for immunoprecipitation to investigate C. trachomatis.

3- The thired swap was used for molecular detection techniques and immersed in plain tube-containing 1 ml of phosphate buffered saline (PBS) transport medium or normal saline for genes detection .

Detection of IgG in Vaccinated Women

Five (ml) of venous blood were taken from 185 vaccinated women. The blood samples were collected in plain tubes and centrifuged for 10 minutes at 2500 RPM. The sera were separated and kept frozen at -20C◦ until used for measurement [13]. the presence of antibodies (IgG) using rapid immunochromatographic card test (Weifang Kanghua/China).

The specimen was transported to the laboratory to the Collage of Sciences at University of Mosul, Mosul,Iraq.

DNA Extraction and PCR Protocol

The collected endocervical swabs from patients were subjected to DNA extraction procedure. It was performed according to protocols recommended by manufacturer (Geneaid/USA). The achieved DNA was stored at 2-8°C for further applications and processing for molecular identification of C. trachomatis using specific primers for detecting three genes (pgp2,pgp3, and chxR), using conventional PCR primers genes and their conditions listed down in Table .(1) and Table.(2).

Table.(1): Primers and product size of gene

Size of gene(bp)	Primerlength	Sequence(5`_3`)	Gene	References
614	21	AGCTGCTGTAATCACCCAGTC	*pgp2-F*	Designed using pick primer program withinNCBI
	21	AAGGGGAACACAAAAGGGGTT	*pgp2-R*	
417	20	CACCGCTTTCTAAACCGCCT	*pgp3-F*	
	20	CCGCTCAAGGACCAGCAAAT	*pgp3-F*	
534	21	GCTACAATCGCTTTGGCATGA	*chxR-F*	
	20	GTCCGCAGACCTTGGTTGTCT	*chxR-R*	

Table.(2):Program and conditions for pgp2,pgp3 and chxR genes

No.	Stage	temperature	time	cycle	References
1	Initial denaturation	95	Min5	1	
2	Denaturation	95	Min 1	3	Determine the temperature using primer3 plus program
3	Annealing		1Min		
	chxR	57			
	pgp3	57			
	pgp2	53			
4	Extension	72	1Min		
5	Final Extension	72	Min5	1	

PCR amplification was confirmed by agarose gel electrophoresis [14]. the electric current was allowed to pass at 70 volt for 50 min. UV trans-illuminator was used for the observation of DNA bands, the gel was photographed using digital camera [15].

Statistical analysis

Frequency distribution of C. trachomatis was introduced in the form of percentage and histograms.

Results

Out of the total 340 women, only 27/340 (7.9%) infection women were diagnosed by Giemsa staining and microscopy to be Chlamydia positive, as shown in Fig.(1),It was based on the presence of inclusion bodies stained in smears from the endocervix which appeared circular oval in shape within the cytoplasm of the epithelial cell, as shown in Fig .(2) while the remaining (313/340) were negative for Chlamydia infection.

Fig.(1): Numbers and frequency of C. trachomatis infection using Giemsa, immunoprecipitation and PCR

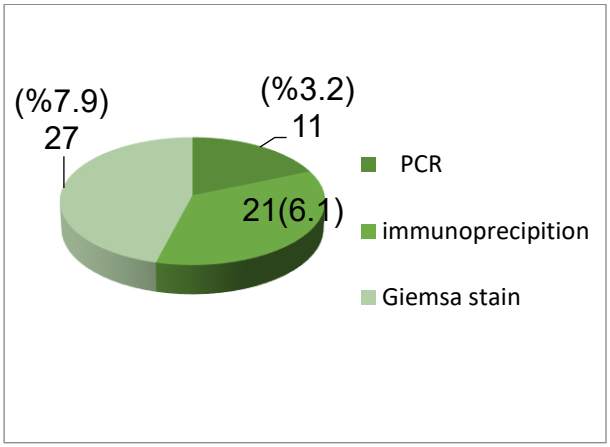
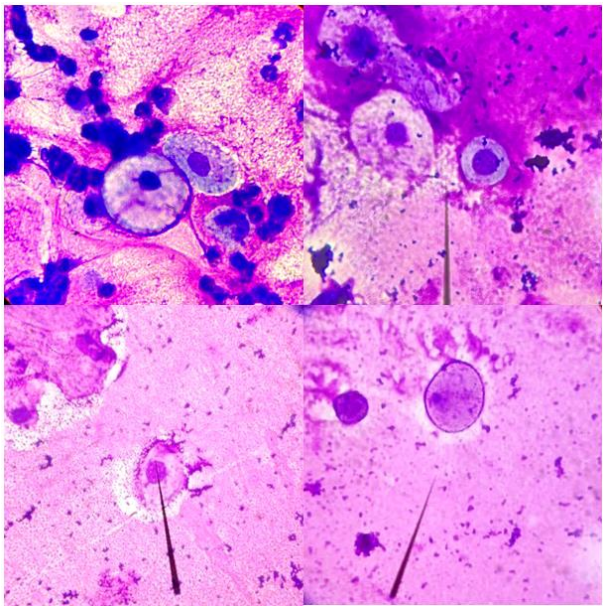


Fig.(2) :Chlamydia trachomatis inclusion bodies staining with Giemsa stain



Using the rapid immunoprecipitation for detection of Chlamydia as antigen in (340) symptomatic and asymptomatic cases, 21 (6.17%) patients were found to be Chlamydia positive, whearas the remaining (319/340) were negative for this bacterium. It is noticed that out of 11/340 (3.17%) of women showed positive results for the three genes using conventional PCR . In women who were infected with C. trachomatis they were strongly linked to tubal EP :11 positive cases out of 27 tubal EP is 5.7 times more common in women who were infected with C. trachomatis[17], as shown in Fig.(1).

Primers for pgp2,pgp3 and chxR genes were designed for the first time in the current study and were specialized for diagnosis C. trachomatis ,all of them yielded approximate results 11/340 (3.2%) at molecular weight 534 bp ,614 bp ,and 417 bp, respectively, as shown in Fig.(3),and Fig.(6).

Fig. 3 The numbers and frequency of the presence of genes pgp2,pgp3,and chxR using primers designed for the first time in the current study

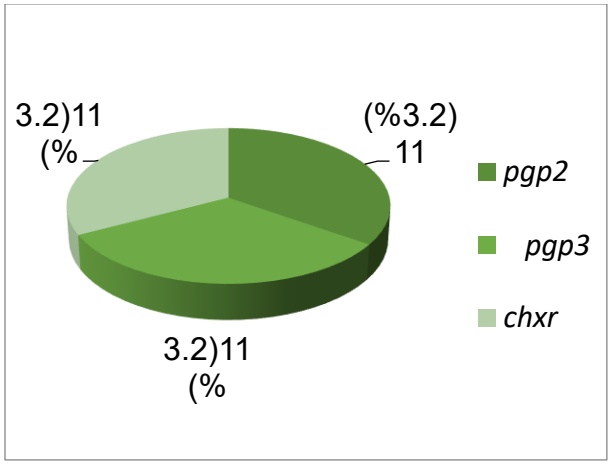


Fig.(4): pgp2 gene PCR products at 534 bp for Chlamydia trachomatis in endocervical swabs among patients with ectopic pregnancy disease. Using 1% Agarose gel electrophoresis at 70 volt for 50 min the pgp2 bands visualized under U.V light after staining with ethidium bromide. L: 1500 bp ladder;positive results lane (1-6) }

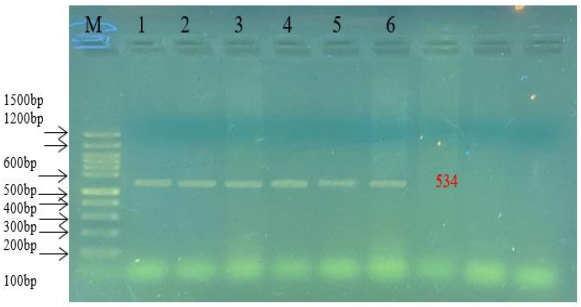


Fig.5 pgp3 gene PCR products at 534 bp for Chlamydia trachomatis in endocervical swabs among patients with ectopic pregnancy disease using 1% Agarose gel electrophoresis at 70 volt for 50 min the pgp3 band visualized under U.V light after staining with ethidium bromide. L: 1500 bp ladder; positive results lane (1-6)

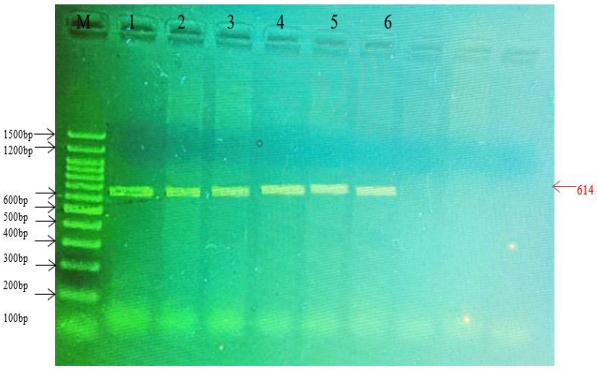
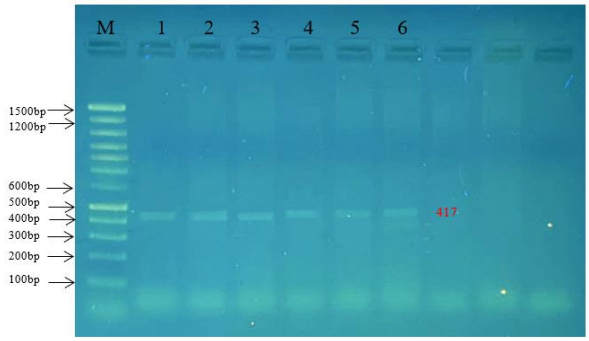


Fig.(6): ChxR gene PCR products at 534 bp for Chlamydia trachomatis in endocervical swabs among patients with ectopic pregnancy disease. Using 1% Agarose gel electrophoresis at 70 volt for 50 min the pgp2 bands visualized under U.V light after staining with ethidium bromide. L: 1500 bp ladder;positive results lane (1-6)



The immunoprecipitation method was evaluated in relation to the presence or absence of (IgG) in vaccinated women under study .Table. (4) shows that the number of vaccinated with SARS-Covid-19 was (182), 6(3.3%)of them were Chlamydia trachomatis positive , 1/6 was IgG positive. For non-vaccinated, it reached (158).

Chlamydia trachomatis infection was detected in 21/158(13.3%). whearas the antibodies was investigated in the serum of the vaccinated women using the immunoprecipitation technique.it was noticed that the presence of immune bodies was observed in one woman from the total.

Table.(3): Chlamydia trachomatis infection and its relationship with SARS-COVID-19 vaccine

Patients	No. (%)			Total
	Chlamydia positive	*Chlamydia* negative	IgG	
Vaccinated	6 (3.3)	176 (96.7)	1(0.5)	182
un vaccinated	21 (13.3)	137 (86.7)	0	158
Total	27(7.9)	313(92.1)	1	340

11 (6.9%) of the latter cases belong to the 158 symptomatic patients and 16 (18.7%) cases belong to the 182 asymptomatic patients as shown in Table 3.

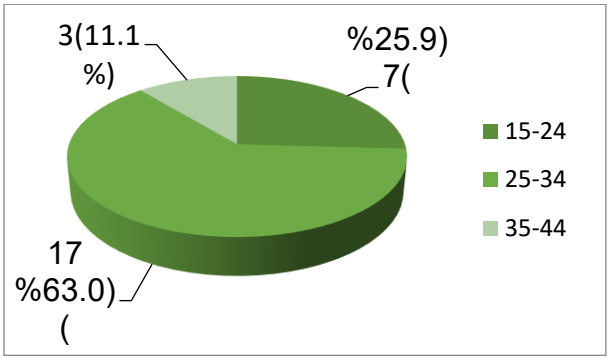
Table (4): Numbers and percentages of Chlamydia infection detected in symptomatic and asymptomatic cases.

Patients	No. (%)		Total
	Chlamydia positive	*Chlamydia* negative	
Symptomatic	11 (6.9)	147 (43.2)	158
Asymptomatic	16 (8.8)	166 (48.8)	182
Total	27(7.9)	313(92.1)	340

Also,the analysis of the frequency distribution of C. trachomatis among age of patients with (EP) revealed that the highest frequency among the women aged (24-34) years was 17/27 (63%), whearas 7/27 (25.9%) for women aged group (15-24)years, and 3/8

(11.1%) for women aged (35-44) years. This means the frequency was about (75%) above age 20 years as compared with (25%) at age 20 years and below, as in Fig.(9).

Fig.(7) Number and frequency of Chlamydia trachomatis in endocervical swabs of woman according to age.



4- Discussion

Generally, the diagnosis of Chlamydia is not easy due to the difficulties in its culturing and its direct identification using special staining techniques [19]. Consequently, patients with Chlamydia infection may suffer from long term sequelae, e.g., (EP).

In this study, we evaluated the diagnostic accuracy of different direct microscopy, immunoprecipitation, and molecular methods to diagnose chlamydial infections among women surffering from symptoms and asymptoms. The cases were diagnosed as having (EP)by the gynecologist and had risk factors for this infection. , using Giemsa stain method and PCR technique as the standard gold.This study was targeted cryptic plasmid by PCR technique,it is useful for the identification of pgp2,pgp3 and chxR genes.

Recently, immunoprecipitation was developed for the detecting of chlamydial antigen and was used in the present study. It provided a rapid and practical way for the detection of Chlamydial infection. In the current study, the prevalence of Chlamydia in Giemsa staining, immunoprecipitation and PCR technique was 7.9% , 6.1% ,and 3.2%) ,respectively.)

The result are in agreement with different studies conducted in the Arabic World in Iraqi,it was(3.4%)[33]while in Jordan,it was (3.9-5%) [25, 26] and in Qatar,it was (5.3%) [27], while in Saudi Arabia,it was (15%) [28]. Additionally, in Iran two separated studies reported that frequency of C. trachomatis was (8.3%) and (22%), respectively [29, 30] in the United Arab Emirates,it was (2.6%) [24]

This distinction in the frequency is related to sample size, age of the participants, population studied with variable socioeconomic factors, along with different practices used in the studies. [30].

5- Conclusions

This study has shown that a greater proportion of women with (EP) had molecular evidence of prior Chlamydia trachomatis infection than women with uncomplicated intrauterine pregnancy. Using the pgp2,pgp3, and chxR genes provesd to be a sensitive and specific method for the identification of C. trachomatis in women. Low frequency of chl amydial infection in association with (EP)among Iraqi female patients might be due to Islamic and Arabic traditions and ethics.

The immunoprecipitation for Chlamydia antigen represented a rapid, informative, and not expensive method for the detection of Chlamydia infection .

Reference

1- Willey, J. M., Sandam, K.M, Wood D.H and Prescott,L.M.(2020) Prescott's Microbiology 24th ed. McGraw Hill.

2- O'Connell, C. M., and Ferone, M. E. (2016). Chlamydia trachomatis genital infections. Microbial Cell, 3(9), 390.

3-Lehr, S., Vier, J., Häcker, G., and Kirschnek, S. (2018). Activation of

neutrophils by Chlamydia trachomatis-infected epithelial cells is modulated by the chlamydial plasmid. Microbes and Infection, 20(5), 284–292. https://doi.org/10.1016/j.micinf.2018.02.007

4- Comanducci M, Ricci S, Cevenini R, Ratti G. 1990. Diversity of the Chlamydia trachomatis common plasmid in biovars with different pathogenicity. Plasmid 23:149 –154. https://doi.org/10.1016/0147-619x(90)90034-a

5-Gong S, Yang Z, Lei L, Shen L, Zhong G. 2013. Characterization of Chlamydia trachomatis plasmid-encoded open reading frames. J Bacteriol 195:3819 –3826. https://doi.org/10.1128/JB.00511-13.

6-Moulder J. W. (1991). Interaction of chlamydiae and host cells in vitro. Microbiological Reviews, 55(1), 143–190.

7-Hou, S., Dong, X., Yang, Z., Li, Z., Liu, Q., and Zhong, G. (2015). Chlamydial plasmid-encoded virulence factor Pgp3 neutralizes the antichlamydial activity of human cathelicidin LL-37. Infection and Immunity, 83(12), 4701-4709.

8-Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. Nature, 415(6870), 389-395.

9-Yang, C., Kari, L., Lei, L., Carlson, J. H., Ma, L., Couch, C. E., and Caldwell, H. D. (2020). Chlamydia trachomatis plasmid gene protein 3 is essential for the establishment of persistent infection and associated immunopathology. MBio, 11(4), e01902-20.

10- Li, Z., Chen, D., Zhong, Y., Wang, S., and Zhong, G. (2008). The chlamydial plasmid-encoded protein pgp3 is secreted into the cytosol of Chlamydia-infected cells. Infection and Immunity, 76(8), 3415-3428.

11-Koo, I. C., Walthers, D., Hefty, P. S., Kenney, L. J., and Stephens, R. S. (2006). ChxR is a transcriptional activator in Chlamydia. Proceedings of the National Academy of Sciences of the United States of America, 103(3), 750–755. https://doi.org/10.1073/pnas.0509690103.

12-Swain, G. R., McDonald, R. A., Pfister, J. R., Gradus, M. S., Sedmak, G. V., and Singh, A. (2004). Decision analysis: point-of-care Chlamydia testing vs. laboratory-based methods. Clinical Medicine and Research, 2(1), 29-35.

13- Abdella, K., Abdissa, K., Kebede, W., and Abebe, G. (2015). Drug resistance patterns of Mycobacterium tuberculosis complex and associated factors among retreatment cases around Jimma, Southwest Ethiopia. BMC Public Health, 15(1), 1-7.

14-Sambrook, J., and Russell, D. W. (2001). Molecular cloning: a laboratory manual., 3rd edn.(Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York).

15-Vendramini, A. A., Lábio, R. W. D., Rasmussen, L. T., Reis, N. M. D., Minett, T., Bertolucci, P. H. F., ... and Payão, S. L. M. (2011). Interleukin-8-251T> a, interleukin-1α-889C> t and apolipoprotein e polymorphisms in Alzheimer's disease. Genetics and Molecular Biology, 34, 1-5.

16-Workowski, K. A., Berman, S. M., and Douglas Jr, J. M. (2008). Emerging antimicrobial resistance in Neisseria gonorrhoeae: urgent need to strengthen prevention strategies. Annals of Internal Medicine, 148(8), 606-613.

17-Rashid, F., Wardeh, M., Das, S., Sattar, M. A., Mollah, A. M., and Chowdhury, S. (2022). Chlamydia trachomatis Infection in Tubal Ectopic Pregnancy. Journal of South Asian Federation of Obstetrics and Gynaecology, 14(2), 101-105.

19-Berman SM, Campbell CH, Geissman K et al. Recommendation for prevention and management of Chlamydia trachomatis infections. CDC, MMWR; (1993). 142/ RR-12: 1-32.

24-Ghazal-Aswad, S., Badrinath, P., Osman, N., Abdul-Khaliq, S., Mc Ilvenny, S., and Sidky, I. (2004). Prevalence of Chlamydia trachomatis infection among women in a Middle Eastern community. BMC women's health, 4, 1-6.

25-Awwad, Z. M., Al-Amarat, A. A., and Shehabi, A. A. (2003). Prevalence of genital chlamydial infection in symptomatic and asymptomatic Jordanian patients. International journal of infectious diseases, 7(3), 206-209.

26- Al Ramahi, M., Mahafzah, A., Saleh, S., and Fram, K. (2008). Prevalence of Chlamydia trachomatis infection in infertile women at a university hospital in Jordan. EMHJ-Eastern Mediterranean Health Journal, 14 (5), 1148-1154, 2008.

27- Al-Thani, A., Abdul-Rahim, H., Alabsi, E., Bsaisu, H. N., Haddad, P., Mumtaz, G. R., and Abu-Raddad, L. J. (2013). Prevalence of Chlamydia trachomatis infection in the general population of women in Qatar. Sexually Transmitted Infections, 89(Suppl 3), iii57-iii60.

28- Kamel, R. M. (2013). Screening for Chlamydia trachomatis infection among infertile women in Saudi Arabia. International Journal of Women's Health, 277-284.

29- Afrakhteh, M., Mahdavi, A., Beyhaghi, H., Moradi, A., Gity, S., Zafargandi, S., and Zonoubi, Z. (2013). The prevalence of Chlamydia trachomatis in patients who remained symptomatic after completion of sexually transmitted infection treatment. Iranian journal of Reproductive Medicine, 11(4), 285.

30- Chabuck, Z. A., Al-Shukri, M. S., and Gatea, A. K. (2016). Molecular Diagnosis of Genital Chlamydia trachomatis in Women with Pelvic Inflammatory Disease. RESEARCH JOURNAL OF PHARMACEUTICAL BIOLOGICAL AND CHEMICAL SCIENCES, 7(5), 2543-2549.

31- Elwell, C., Mirrashidi, K., and Engel, J. (2016). Chlamydia cell biology and pathogenesis. Nature Reviews. Microbiology, 14(6), 385–400. https://doi.org/10.1038/nrmicro.2016.30

32-Zigangirova, N. A., Rumyantseva, Y. P., Morgunova, E. Y., Kapotina, L. N., Didenko, L. V., Kost, E. A., Koroleva, E. A., Bashmakov, Y. K., and Petyaev, I. M. (2013). Detection of C. trachomatis in the serum of the patients with urogenital chlamydiosis. BioMed Research International, 2013, 489489. https://doi.org/10.1155/2013/489489

33-Al-Jammally, M. M., and Al-Taii, H. A. (2020). Comparison of Methods for Detection of Chlamydia trachomatis in cervical from women in mosul city. Journal of Education and Science, 29(1), 121-134

34- Sheetawy, A. Z., and Abdulla, Z. A. (2007). Detection of chlamydia and other bacteria in cervicitis. Ann. Coll. Med. Mosul, 33(1and2), 26-34.

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