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Efficacy of Some Laboratory Samples and Techniques in Detecting *Chlamydia trachomatis* Infection among Women in Kirkuk Province

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Abstract

Chlamydia trachomatis (CT), Trichomonas vaginalis, Neisseria gonorrheoae and other viruses are common causes for women urogenital discharges. The efficacy of laboratory methods and samples were aimed to reach final accurate, rapid method for diagnosis of CT.A total of 185 women urogenital samples tripled number from endocervical swabs, high vaginal swab(HVS) and urine deposits were collected from women in child bearing ages, involving 166 with urogenital discharges and the rest number as control (without discharge). For each specimen direct wet preparation, gram staining, rapid lateral immune-chromatography assay (RILCA) and ELISA technique were applied. The all rate of urogenital infection was 33, 23% Bacterial infection contributes high rate 55.22%, followed by CT 41.96%, fungi 22.23% and 12,58% for *Trichomonas vaginalis. Gardnerella vaginalis* and *Moblincus* species are dominant bacteria in HVS versus to CT in endocervical swabs. The rate of CT infection was high in specimen with yellowish discharge, RLICA technique is with efficacy to demonstrate CT than by using direct, gram staining, culturing and ELISA technique. The rate of CT was higher in the second and third period of gestation than in the first period. Young, aged women (15 to 30 years) are highly at risk for CT infection than elderly women. The relationship between women, abortion, parity and CT frequency was not significant. Pelvic inflammatory disease in high correlation to CT infection. Cervical erosion, vaginal itching, fever and dyspareunia are a dominant feature during women CT infection. Conclusions: The rate of STD was highest among young aged women particularly CT infection. Endocervical swabs and RLICA technique have had high efficacy in demonstrating CT than other lab methods. The relationship between genital discharge color gestational periods and CT existence was significant. Whereas it was negative in regard number of women abortions and women parity. This study was preliminary study that required further studies to a sure real rate of CT and other STD.

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Keywords

Chlamydia; Trichomonas; Gardnerella; Endocervical; RLICA; ELISA

Introduction

Sexually transmitted disease (STD), is a major problem facing individuals who conceiving child (European Centre for Disease Prevention and Control, 2014). It involves different etiology such as, protozoan like trichomoniasis that causes vaginitis in both women and men by the protozoan parasite called *Trichomonas vaginalis*. *Human herpes simplextype-2*, *Human papilloma* viruses, Hepatitis B and Human immune-virus (HIV) are second type the cause for STD. Whereas *Treponema palladium* (Syphilis causative agent),

gonorrhea (GC) and *Chlamydia trachomatis* (CT) are examples for bacteria causing STD (Ángel-Müller *et al.*, 2012). The genus, *Chlamydia* involves obligate intracellular parasites and they have not been propagated out of the host cells. DNA analysis reveals four main species causing different types of diseases. But regarding CT, three serovar have been found to affect human being; they are serovars A-C cause trachoma, D-K cause non-gonococcal urethritis, muco-purulent cervicitis and inclusion conjunctivitis. In addition to L 1-3 cause lymph-granuloma venereum (LGV) (Ryan *et al.*, 2010).

There are thought to be 50 million new cases world-wide annually. High risk 16.4 % has been reported among people in United Kingdom. Approximately 700 000 new cases of *Chlamydia* infection are reported each year in United States. The prevalence of *Chlamydia* in adolescent populations ranges from 6 to 12 % (Higgins *et al.*, 1998).

Genital infection with *C. trachomatis* is asymptomatic in 50-88% of Colombian women (Higgins *et al.*, 1998) and is most common in young women (Molano *et al.*, 2005). Untreated infections can cause pelvic inflammatory disease (PID) (Adams *et al.*, 2004), ectopic pregnancy (Risser and Risser, 2007), and subfertility (Van Valkengoed *et al.*, 2004).

Risk factors for *C. trachomatis* infection include young age, having more than one sexual partner, and recent change of partner, (Land *et al.*, 2010; Pinto *et al.*, 2011). Chlamydial urethritis is manifested by a thin urethral discharge, dysuria, infection of the uterine cervix may produce vaginal discharge, but most often are a symptomatic. Salpingitis and pelvic inflammatory disease (PID) may produce when the infection is ascended. It has been found in 5 to 30 % of infected women (Detels *et al.*, 2011). Sterility and ectopic pregnancy are the important features of chronic or repeated CT infection (Gottlieb *et al.*, 2010).

Women under 20 years of age are the most infected, this is thought to be due to anatomical differences in cervix of younger women, use of contraceptives particularly oral type and higher sexual activity also increases the risk of infection (Bebany, 2008).

Clinically CT difficulty diagnosed because it required especial procedure like cell culture and most of the culture failures might be due to the transmission because CT organism will die within a short period after discharge from the infected person. While the color of the discharge and Gram staining had value in classification of urogenital secretion in non-gonococcal. But to obtain an accurate result, it requires an expert laboratory technician and laboratory facilities. In recent decades the rate of STD in Iraq and particularly in Kirkuk Province was increased and according to available data studies to find the rate of the causative agents for STD, particularly among women in Kirkuk Province are rare, except the study carried on during 2008 by (Bebany, 2008). Who found the following rates 19 %, 18.5%, 14%, 11.5% and 4.5 % of *Trichomonas vaginalis, Gardnerella vaginalis, Chlamydia trachomatis, Candida albicans* and *Neisseria gonorrhea* respectively. Furthermore, in recent the diagnosis of CT is mostly by applying PCR technique which was expensive and long-time consume, so this study was planned to estimate the rate of STD, classification of infectious agents, searching for the most efficacy modes of sampling and compression between direct microscopy, ELISA and rapid lateral immune- chromatography assay (RLICA).

Materials and Methods

Study design and population

From 1st of February 2018 to 1st of August 2018, a cross sectional study was carried out on 185 women, attending to obstetric and gynecological department in Azadi Teaching Hospital in Kirkuk city. In addition, from women who attend into two private clinics in the Kirkuk medical street. They were aged from 15 years to 55 years. Their compliances involve; cervicitis, vaginitis, urethritis, bad odour, itching, back pain, dyspareunia and other signs and symptoms. Study population classified into 141 non-pregnant women, 20 pregnant women, and 25 women without any signs and symptoms (control group).

Participants provided signed informed consent and completed a 10-minute questionnaire administered by trained health professionals who attended the services. Exclusion criteria included administration of systemic or topical antibiotics within one month prior to sampling.

Ethical approval

This study approved by the Medical Ethics Committee in College of Medicine, Kirkuk University. An informed consent was taken from the participants subsequently they presented for clinical examination and urogenital discharge sample collections.

Samples collection

From each participant three different following samples were collected as follows; midstream or clean catch procedure was advised for urine collection, which was collected in sterile fitted tight screw lid to avoid sample leakage and contamination. This specimen was transferred in a cool box and transferred to a lab for sample processing (Knox *et al.,* 2002). While vaginal and endo-cervical discharges were collected by insertion of a Cuscos bivalve speculum and the discharge from the posterior vaginal fornix and also from endo-cervix using sterile cotton-tipped swabs. These samples were socked in to another tube containing transport medium. As soon as the samples were collected, they transported at once to the lab for processing (Centers for Disease Control and Prevention, 2014). In case the delay of sample arrival to the lab the samples were transported on ice packs and stored at -20ºC until processed.

Laboratory procedures

Urine sample processing

Each urine samples were divided into 2 containers under sterile procedure; the standard loop-ful from the first portion was inoculated on routine culture media for detecting pathogenic bacteria causes urinary tract infection (UTI) especially the members of *Enterobateriaceae* and for gonorrhea (Vandepitte *et al.,* 1999). Whereas the second portion was checked macroscopically for color, odour and for any inclusion bodies. Followed by dip-stick chemical examination for detecting pH, sugar and albumin. For microscopy a fresh drop of urine deposit after centrifugation (3000 RPM for 5 minutes) was examined for red blood corpuscles (RBCs), polymorph neutrophil cells (PMNs), bacteria, *Candida*, clue cells, urine crystals and *Trichomonas vaginalis* (Laposata, 2010). A second drop of the deposit was distributed and fixed on a microscopic slide. Stained by gram staining procedure for detecting any microorganisms with emphasis on *Neisseria gonorrhea* (World Health Organization, 1999).

Chlamydia trachomatis in urine was detected by using a specific kit purchased from Biozek company-Netherland. The procedure applied according to manufacture company leaflet; briefly the procedure involved the transferring of 10 ml of morning, first void in a test tube for centrifugation spin up to 3000 rpm for 15 minutes. The supernatant was discarded; 200 µl of reagent B was added on the urine deposit, agitated for one minute until the suspension is homogenous. To the content of the test tube about 300 µl from the reactant A in vertical position was added, the tube was rotated to mix solution. The tube and the content was left for 2 minutes 3 drops of the extracted solution (about 100 µl) was transferred in the pit of the sample (S) of the card and start timer. Air bubbles was avoided by knocking the strip gently. The red bands in test line and control lines were read within 10 minutes and considered as positive for CT. Red band formation in control line only means negative for CT (Sanders *et al.,* 1994).

For swabs with female cervical samples or high vaginal swabs: 5 drops of reactant A (about 300 µl) were transferred in a test tube for the extraction of the sample. Reactant A has no color. Immediately the swab was soaked in the solution and the swab was rotated for two minutes and pressed of the test tube. The content of the tube was left for 2 minutes. Reactant B up to indicated level about 220 µl was in the sample extraction test tube rotated 15 times until the solution converted cloudy. The results positive or negative were detected as it was described for urine samples within 10 minutes (Bebany, 2008).

The second swab from each of endo-cervical and high vaginal swabs was observed carefully and seeded on two blood agar media(one aerobically and the other under CO2 using a candle jar). In addition to streaking on MacConkey medium for G-ve bacteria isolation. The isolated bacterial colonies were checked using different bacteriological tools for identification. For each swab; direct microscopy was done using Gram staining for detecting gonorrhea, and other bacteria. In particular from high vaginal swab; direct wet preparation was prepared for detecting *Trichomonas vaginalis* trophozoites. Moreover. The color, odour and pH of each specimen were checked. For *Gardnerella vaginalis*, Whiff test (Liberation of amine form high vaginal specimen) was done, briefly the method is a simple chemical method started by rubbing the small portion of a vaginal discharge on clean microscopic slide then 1 to 3 drops of the clear KOH solution was added to it. Fishy odour liberation means positive (Hussein, 2009).

Chlamydia trachomatis IgM antibody detection (ELISA)

ELISA kit was purchased from Cortez Diagnostics, Inc USA. This lab test was done according to manufacture company described procedure applied as follows: the sera were diluted 1:40 by adding 5 µl of the test samples

was added to 200µl of sample diluent, 100 µl of diluted sera, calibrator, and controls were transferred by automatic micropipette into the appropriate wells. For the reagent blank, 100 µl of the sample diluent solution was transferred into 1A well position. The holder was tapped to remove air bubbles from the liquid and mix well. Incubated for 30 minutes at room temperature. The contents of each well was discarded and about 350 µl of wash solution was added into each well, except A1 (the blank). Washing process was repeated for three times, dried and 100 µl of enzyme conjugate to each well was despised and incubate for 30 minutes at room temperature. Enzyme conjugate was removed from all wells. Three times of washing were applied using a wash buffer. The wells were dried, then 100 µl of TMB Chromogenic Substrate to each well was added and incubated for 15 minutes at room temperature in a dark place. The reaction was stopped by adding 100 µl of Stop solution to each well. Air bubbles were removed from each well before reading the optical density (O.D.) at 450 nm with ELISA micro well reader. Results interpretations: negative: IgM index of 10 IU/ml or less are sero-negative for IgM antibody. Positive: IgM index of 11.00 IU/ml or greater. Equivocal: IgM index of 10.1 – 11 IU/ml are equivocal (Salman, 2014).

Statistical analysis

All obtained data have been organized in tables and the statistical analysis was performed using statistical analysis system (SPSS); version 16. (SPSS Inc. Chicago IL. USA). Frequency and percentage were used with qualitative data. Z-test and Chi-square were used to compare frequencies.

Results and Discussion

The following table 1. Is summarizing the general information and available date extracted from the special questionnaire for each woman participates in the current study.

The following microorganism were found in the specimen: 209 positive samples 41.96% was recorded for *Chlamydia trachomatis*, followed by 275 positive samples 55.225 for other pathogenic bacteria, 114 samples, 22.89 % for fungi mostly they are *Candida* species and 64 positive samples for *Trichomonas vaginalis* was recorded, the rate was 12.85%, P<0.05. Regarding to all microorganisms in relation to types of laboratory samples reveal significant relation P<0.05, via which high rate of organisms 53.21 % was recorded in endo-cervical swabs versus to 138 samples, 31.76 % was recorded in urine samples, P<0.05 (Table 2).

The relationship between types of microorganisms distribution according to laboratory sample significant and arranged in table 3, through which; endo-cervical samples reveals 15 different bacterial species in addition to Chlamydia trachomatis antibody; the rate was 53.01%.Whereas only 10 bacterial species were recorded, the rate was 43.97%. Contrary to 11 species in the urine samples, the rate was 26.50%. *Gardnerella vaginalis* recorded in high rate 21.68 % in high vaginal samples compared to 9.63 % in endo-cervical swabs. *Moblincus* species as Curved bacilli was recorded 5.41 % in endocervical swab compare to1.80% in HVS. Sexually transmitted bacteria such as *Staphylococcus saprophyticus* and *Streptococcus agalactiae* were recorded in high rates in endocervical swabs, the rates were 5.42% and 4.83 % respectively. *Listeria monocytogenes* was recorded in all 3 specimens with high frequency in endocervical swabs 9.63% versus to 4.81% and 2.40 % in both HVS and urine deposits respectively; p<0.05. Moreover *Niesseria gonorrhoeae* was recorded as all rate 3.41%, this rate was distributed into 6.62% in HVS compared to 2.40% and 1.20% in endocervical swabs and urine deposits respectively. According to the type of laboratory samples the following rates of *Chlamydia trachomatis* antibodies were obtained: 62.65 % in endo-cervical samples followed by 57.84 % in high vaginal swabs; whereas 24.69 % as low rate was recorded in urine samples, P <0.05 (Table 4).

The efficacy of different laboratory techniques was shown in table 5; via which Rapid lateral immune-chromatography assay (RLICA) was superior on the other laboratory tests used for demonstrating *Chlamydia* antibodies in all 3 samples. The following rates were obtained 38.87 % for RLICA compare to 3, 22 % for ELISA IgM, P<0.05. Regarding types of samples; high rate of *Chlamydia trachomatis* 26.12 % was recorded from endo-cervical samples followed by 19.83 % and 8.22 % for HVS and urine deposit samples respectively, P<0.05.

Color of urogenital discharge and *Chlamydia trachomatis* frequency was tabulated in table 6; which reveal significant relationship P<0.05; within which white discharges samples reveal high rate 18.27 % of *Chlamydia* antibodies followed by 13.05 % in yellowish discharges. Contrary to transparent discharges that reveal lowest rate 2.08 %.

Considering patient ages (Table 7) is showing that, young aged patient (15 to 30 years) samples have high rate 74.69 % of *Chlamydia trachomatis* antibody compared to 4.21 % in samples for patients aging from over than46, P<0.05.

The correlation between types of laboratory samples and *Chlamydia* frequencies reveal the followings: the rate of infection was higher in endocervical swabs and High vaginal swabs; the rates were 57.22% and 51.20 % respectively, versus 17.46 % in urine deposits, P<0.05.

According to women gestational periods; *Chlamydia* antibodies were found in specimens belongs to women in gestational period, followed by 33.33% in the third period and 22.22% in the first period of gestation, P<0.05. while according to laboratory samples the rate of *Chlamydia* was high 50% in endocervical swab, compared to 30 % and 15% in HVS and urine deposits samples, p<0.05. In general *Chlamydial* infection was higher in endocervical swabs than the rest samples in addition to high frequencies in third and second periods of gestation (Table 9).

The relationship between number of child conceiving, number of women abortion and frequency of *Chlamydia trachomatis* antibody was obvious in table 9, via which the relationship in the former was significant p>0.05; through which women with no children have 51.20 % of Chlamydia infection compared to 62.99% among women with children. Moreover, women with abortions, which exert significant variance between the specimens belongs to aborted women 54.61 %which was higher than 13.38% in the specimens from women with no abortions.

Sexually transmitted diseases (STD) was not taken in consider in Iraq, just that some researches showing the rate infection using very old methods or the case itself was detected by the physicians according to sign and symptoms. Therefore the current study was the first in Kirkuk province that deals with new rapid laboratory techniques for detecting STD among childbearing women, particularly *Chlamydial* infections. In general the rates of all microorganisms STD 33.23% were high as the bacterial infection rate 55.22%, *Chlamydia* 41.96%, fungi 22.89% and 12.58% for *Trichomonas vaginalis.* These rates were reflection the degree of spreading of theses microorganisms among women in this Province, actually had impacts on both partners in any family and also the impacts on the pregnancy outcomes particularly when the fetus delivered normally. A consistent prevalence of laboratory confirmed STD was recorded in Egypt 71.6% (Ali *et al.,* 1995). A relatively higher prevalence 85% were found in reports from Ibadan in Nigeria (Kehinde and Lawoyin, 2005). However, a comparatively slightly higher prevalence 39% was also reported in Ibadan in Nigeria (Okonko *et al.,* 2012).

This is due to the fact that the prevalence of STD agents changes with time, and the distribution of STD agents varies from place to place. Moreover 15 different bacterial species isolate in urogenital of women place the outcomes of the pregnancy on risk particularly CT, *Neisseria gonorrhoeae, Listeria monocytogenis* in addition to these pathogens on women's health in this Province, the causes to these cries; that's mostly due to that most of peoples in Iraq live under poor hygienic condition and low levels of sanitation. Poverty, continuous wars on Iraq, instability after 2003 in addition to economic sanction previously persist for more than 12 years, therefore all these factors can explain why the rate of STD was higher among women in Kirkuk (Salman, 2014).

In the current study the high rate 10.44% of *Gardnerella vaginalis* record was high compared to that recorded by (Bebany, 2008) and by (Hussein, 2009) in the same province. Actually Bacterial vaginosis refers to reduction in the number of the lactobacilli in the vagina that maintain the pH of the vagina, so in this condition women most often suffers from bad fishy odor secretion, burning sensation itching and during the third trimester, may give rise to preterm labour and still birth sequels (Hussein, 2009) Variances in the rates might be due the size of lab samples, techniques, displacing of peoples after 2014 in Northern of Iraq, lack of medicine and continuous water interruption in addition to malnutrition that had strong impacts on the immune system. The records of *Staphylococcus saprophyticus* and *Moblincus* (Curved rods revers to *Gardnerella* which was straight). The former means that this normal flora were pathogens in urogenital and has a part in the rate of STD. whereas the later existence refer to the fact" vaginosis among women in Kirkuk Province regarding bacteria not restricted to *Gardnerella vaginalis* alone". Both records were for the first time in Kirkuk – Iraq.

It has been known that a high rate of women's pelvic inflammatory diseases (PID) was mostly due to *Chlamydial* infections; this fact was obviously seen in the current study; as, 160 women have had PID and contribute in the high percentage within the all rate 41.96% of Chlamydial infections.

Table.1 General information's and available data for patients enrolled the study

Parameters	Available data
Total patients number	166
Non-pregnant women	141
Pregnant women	25(7 1st trimester, 8 in 2nd and 10 in 3rd trimester)
Residency	140 from urban area and 26 from urban area
Women state	160 married, divorced(4) and widow (2)
Parity	Parity 148 and non-parity 18
Abortion	Non-aborted(100) and 66 women with multiple abortions
Education levels	117 house makers,Bachelor (24), diploma(14), students (9) and children(2)
Women gestation outcomes	Normal(144),caesarian (19), ectopic (1) and hydatid mole 1
Infertility	Infertility (6) and 160 fertile.
Surgical operations	Caesarian (19), appendicitis (9), gall bladder(3), tube ligation(2), hysterectomy(2) and eye (2)
Contraception's	Pills (2), IUCD (27), condom (21),other modes(60) and non-use contraception 46.
Diseases	Hypertension (6), hypotension (7), DM(5), migran (2) and asthma (1).
Males (women husbands)	27 husbands with urogenital problems
Pelvic inflammatory disease	150
Back pain	161
Leon pain	159
Genital discharges	158 with discharges and 8 women without discharges.
Fever	Feverish women (73) and non-feverish (93)
Skin spots	No spots(128), with skin spots (38)
Dyspareunia	Positive (83)
Bleeding	Positive 31
Uro-Genital itch	Positive 114

Table.2 Types of microorganisms according to urogenital infections

Types of microorganisms	*Chlamydia antibodies*		*Trichomonas vaginalis*		Bacteria		Fungi		Total Microorganisms	
Lab samples	No. Positive	%	No. Positive	%	No. Positive	%	No. Positive	%	No. Positive	%
Endo-cervical	95	57.22	0	0.00	110	66.26	60	36.14	265	53.21 *
High vaginal	85	51.20	36	21.68	85	51.20	33	19.87	239	47.99
Urine deposits	29	17.46	28	16.86	80	48.19	21	12.65	158	31.76
Total	209	41.96	64	12.85	275	55.22	114	22.89	P<0.05	

*P<0.05. The all rate of microbial infection was 33.23%.

Table.3 Common microorganism's distribution according to laboratory samples

Types of samples	Endo-cervical		High vaginal		Urine deposits		Total	
Types bacteria	No.+ve	%	No.+ve	%	No.+ve	%	No.+ve	%
Acinetobacter spp	3	1.80	0	0.0	0	0.0	3	0.60
Escherichia coli	2	1.20	0	0.0	8	4.80	10	2.00
Enterobacter cloacae	5	3.01	0	0.0	5	3.01	10	2.00
Gardnerella vaginalis	16	9.63	36	21.68	0	0.0	52	10.44 *
Klebsiella spp	2	1.20	0	0.0	2	1.20	4	0.80
Proteus spp	3	1.80	0	0.0	3	1.80	6	1.20
Neisseria gonorrhoeae	4	2.40	11	6.62	2	1.20	17	3.41*
Listeria monocytogenes	16	9.63	8	4.81	4	2.40	28	5.62*
Mobiluncus spp	9	5.42	3	1.80	0	0.0	12	2.40
Mycoplasma spp	0	0.0	1	0.60	0	0.0	1	0.20
Staph aureus	6	3.60	4	2.40	6	3.6	14	2.81
Staph saprohyticus	9	5.42	1	0.60	3	1.80	12	2.40
Strep. agalactiae	8	4.80	5	3.01	2	1.20	15	3.01
Strept faecalis	2	1.20	1	0.60	4	2.40	7	1.40
Strept pyogenes	3	1.80	3	1.80	5	3.01	11	2.20
Total	88	53.01	73	43.97	44	26.50	205	41.96

*P<0.05

Table.4 Frequency of *Chlamydia trachomatis* according to type of samples

Samples	No. Positive	% positive	No. Negative	%
Urine	41	24.69	125	75.31
High vaginal swab	96	57.84	70	42.16
Endo-cervical swab	104	62.65	62	37.34
Total	241	48.39	257	51.61

Total examined samples for 3 types =498.

Table.5 Incidence of *Chlamydia trachomatis* according to lab. Techniques

Lab. samples	Endo -cervical swab		High vaginal Swab		Urine deposit		Total	
Lab. techniques	No. positive	%	No. positive	%	No. positive	%	No +ve	%
Direct gram staining	23	13.85	9	5.42	1	0.60	33	5.32
RLICA	104	62.65	96	57.84	41	24.69	241	38.87*
ELISA IgM	10	8.02	6	3.61	4	2.40	20	3.22
ELISA in serum	25	17.36	12	8.33	5	3.47	42	6.77
Mean all rates	162	26.12 *	123	19.83	51	8.22	336	54.19

Total serum number tested by ELISA=144. Total proceeded lab tests= 620. *P<0.05.

Table.6 Relationship between color of uro-genital discharges and *Chlamydia trachomatis* occurrence

Samples / Color of discharges	Endo -cervical Swab No. positive	%	High vaginal Swab No. positive	%	Urine deposit No. positive	%	Total No +ve	%
Creamy	9	5.42	10	6.02	3	1.80	22	4.41
Green	9	5.42	8	4.81	4	2.40	21	4.21
Transparent	5	3.01	4	2.40	1	0.45	10	2.08
White	44	26.58	36	21.68	11	6.62*	91	18.27*
Yellow	28	16.86	27	16.26	10	6.02	65	13.05
Total	95	57.22 *	85	51.20	29	17.46	206	41.96

***p<0.05**

Table.7 Distribution of Chlamydia trachomatis according to patients age

Lab. samples / Age groups/ years	Endo -cervical swab No. positive	%	High vaginal Swab No. positive	%	Urine deposit No. positive	%	Total No +ve	%
15-30	56	58.94	45	52.94	23	79.31	124	24.89
31- 45	37	38.94	35	41.17	6	20.68	78	15.66
46 and above	2	2.17	5	5.88	0.0	0.0	7	1.41
Total*	95	57.22	85	51.20	29	17.46	209	41.96

*p<0.05.

Table.8 Frequency of *Chlamydia trachomatis* according gestational periods

Type of Samples / Gestational periods	Urine deposits No. Exam	No +ve	%	High vaginal swabs No. Exam	No +ve	%	Endo-cervical swabs No. Exam	No +ve	%	Total No. Exam	No +ve	%
First trimester	9	1	11.11	9	1	11.11	9	4	44.44	27	6	22.22
Second trimester	4	0	0.00	4	4	100.00	4	2	50.00	12	6	50.00
Third trimester	7	2	28.57	7	1	14.28	7	4	57.14	21	7	33.33
Total	20	3	15.00	20	6	30.00	20	10	50.00	60	19	31.67

*P<0.05. The rate of Chlamydia infection among pregnant women was 12.03 % from the all rate 41.96%.

Table.9 Distribution of *Chlamydia trachomatis* antibodies in relation to parity and abortions

No. children	No. Exam	%	Positive No	%	No. abortions	No. Exam	%	Positive No	%
No children	39	23.50	20	51.20	No abortion	127	76.50	17	13.38
One	21	12.65	16	76.19	One	15	9.03	12	80.00
Two	43	25.90	28	65.11	Two	16	9.64	5	31.25
Three	18	10.84	9	50	Three	6	3.62	4	66.66
four	33	19.87	19	57.57	More	2	1.21	1	50.00
>4	12	7.22	8	66.66	Total abortions	39	23.49	22	54.61*
Total	127	76.50	80	62.99					
All Total	166	100	100	60.02	All Total	166	100	61	38.74

*P<0.05

Therefore, to reduce the rate of CT infection programs should be applied by Kirkuk health directorate on women, because designed programs to screen and treat CT have 2 purposes: to reduce transmission to partners and thereby lower prevalence and the number of future infections, and to prevent PID and further squeal in the women found to be infected (Low, 2007; National Chlamydia Screening Programme, 2011). Untreated CT infection usually clears within 1.5 years (Althaus *et al.*, 2010; Price *et al.*, 2013), so women who are diagnosed through screening have already "survived" a period of infection without developing PID. The randomized controlled trials of screening and treatment versus no screening can provide direct information on the proportion of CT-caused PID that can be prevented in a population with *prevalent* infection (Scholes *et al.*, 1996). However, it is the proportion of preventable PID in women with *incident* infection that is required to evaluate programs designed to lower infection rates (Low *et al.*, 2009).

According to the laboratory samples employee, high rate of CT belongs to endocervical swab higher than HVS and urine deposits refers to the fact that the endocervical region is the habitat for CT as the pH scale was slightly higher than HVS (Smith and Angarone, 2015). On the other hand HVS infection in the second grade after endocervical swabs most often due to descending the discharges from endocervix and the cases were more sever, because it has been found that the acid pH of the vagina is not proper for propagation of CT (Nourollahpour Shiadeh *et al.*, 2016). Regarding the lowest rate of CT 24.69% in urine deposits despite of long centrifugation to remove pus cells, crystals, urine *Candida* to omit technical errors, this might be related to urine pH as it was alkaline during bacterial infection including CT, this phenomena may had impact to produce false negative that had role in reducing the rate of CT during urine examination. This finding was not agreed that recorded by (Haugland *et al.*, 2010) in Netherland whom they extract that urine sample was proper for the diagnosis of CT.

The efficacy of different laboratory techniques RLICA exerting 38.87% higher rate than Gram staining and ELISA (in genital swab and serum), may explain the high affinity of CT interaction with antibodies on chromatography pad and this technique is more sensitive to CT than ELISA and Gram staining that in both washing processes may remove anti-CT, so obtaining low rates of positivity than RLICA. Furthermore correlation between lab techniques and lab samples as endocervical swab samples reveal high rates of CT among women. Four sets of tests for all patients would have given a stronger design resulting in highest precision in our estimates, as there is a possibility of a *C. trachomatis* diagnosis, even if both urine and HVS are negative contrary to the positivity of endocervical swabs. These finding did not agree that recorded by (Jensen *et al.*, 2004). Low rates in gram stained smears mostly related to CT itself as this bacterium was obligated intracellular and poorly gram stained microorganisms (Centers for Disease Control and Prevention, 2014). Whereas the differences in the rate of CT using ELISA techniques as in serum higher than swab samples, in the former might be due to using serum which may reveal cross antibody reaction with other *Chlamydia* species or other intracellular bacteria such as *Rickettsia* species. Contrary to an excess wash with ELISA buffer in the

latter (genital swabs) may influence and remove CT IGM antibody and give rise low rate of CT (Salman, 2014).

The vagina, ectocervix and endocervix all are susceptible to various pathogens, depending on the type of epithelium present and other factors in the microenvironment. The columnar epithelium of the endocervix is susceptible to infection with *Neisseria gonorrhoeae* and *Chlamydia trachomatis. Herpes simplex virus* may infect both types of epithelium. Vulvovaginal candidiasis is characterized by pruritis and a curd like discharge. Vaginal trichomoniasis is associated with a copious yellow or green, sometimes frothy, discharge (Yusuf *et al.,* 2011). The highest rate of bacteria revealed in whitish discharges, particularly positive for *Gardnerella vaginalis* and the bad odor is mostly due to liberation of an amine group at the end of metabolism of this type of bacterial infection. While yellowish and translucent discharge association with CT infection might be attributed to the action of inclusion bodies in the cervix producing erosions and cell infiltration (Goldman and Green, 2008). Regarding greenish-creamy discharge mostly found in specimens positive for trichomoniasis, this might be attributed to co-existence of other chromo-bacteria producing color such as *Pseudomonas* and achromo-bacteria (Spence and Melville, 2007). The second interpretation; it might be due to pus cells association trichomoniasis, these cells may convert nitrate to nitrite.

Considering the women age and CT frequency in urogenital specimens, high rate of CT among women aging from 15 to 30 years, might be attributed to sexual activities, low experience in sexual style, their husbands have a urinary tract infection (UTI) including CT bacteria. While the rate of infection 41.17% as higher rate in HVS compares to endocervical and urine samples might be interpreted to coexistence of CT with other pathogens such as Candidiasis, gonorrhea and trichomoiasis.

Furthermore the low frequency of CT among women aging over 46 years in current study cannot be taken to consider because the number of women participate in the study was few (only 7 women). So further study using high number of samples with this group of women required to prove low discharges 1.41 %. Women age in relation to CT rate in the current study was in agreement with those reported in the USA and Kirkuk-Iraq by (Bebany, 2008; Centers for Disease Control and Prevention, 2015) respectively.

High rates of CT during second and third gestational periods were prone to the opportunity of abortion, stillbirth, low weight of premature and after delivery CT complications such as respiratory or conjuctival infections. From the results in the table 8, the rate of CT 22.22% also high and reflecting community contamination with a symptomatic CT infections. This finding was in agreement with that recorded by (Sweet *et al.*, 1987; Al-Shimetry, 2006).

Conclusions: The rate of STD was highest among young aged women particularly CT infection. Endocervical swabs and RLICA technique have had high efficacy in demonstrating CT than other lab methods. The relationship between genital discharge color gestational periods and CT existence was significant. Whereas it was negative in regard number of women abortions and women parity. This study was a preliminary study that required further studies to a sure real rate of CT and other STD.

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