



Screening for Chlamydia Trichomatis by PCR and ELISA in Aborted Women in Wasit Province

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Abstract

Sexually transmitted diseases (STDs) indicates many types of clinical syndromes and infections caused by pathogens that can be acquired and passed by sexual contact. The current study which was conducted at privet clinics of Gynecology obstetrics doctors mainly, and at Al Kut hospital in Wasit Province. The samples of the current study consist of blood samples, urine sample from each patient. 100 patients with history of abortion. Urine samples were kept for later DNA extraction. Blood samples were analysed for the detection of Chlamydia trichomatis antibody. The results of the Chlamydia trichomatis antibody were positive in 7% in women with abortion. PCR results were more specific and accurate for the detection of the antigen DNA, 17% Chlamydia trichomatis.

Keywords: sexual transmitted infections, abortion, chlamydia trichomatis, PCR, ELISA.

الكشف عن بكتريا داء المشعرات للنساء المجهضات من خلال فحص PCR وفحص ELISA في محافظة واسط

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الخلاصة

الامراض المنقولة جنسياً تتضمن عدة انواع من المتلازمات والالتهابات التي يكون المسبب فيها جرثومة تنتقل خلال الممارسات الجنسية المختلفة ركزت هذه الدراسة التي اجريت في عيادات النسائية والعمم بصورة اساسية ، وفي مستشفى الكوت للنسائية والاطفال في محافظة واسط ، على جمع 100 عينة دم وادرار من النساء اللواتي تعاني من الاجهاض مرة واحدة او اكثر خلال حياتها تم حفظ عينة الادرار لوقت لاحق من أجل استخلاص . DNA عينات الدم خضعت للبحث عن الاجسام المضادة للبكتريا داء المشعرات . نتائج البحث عن الاجسام المضادة اوضحت ان 7% من النساء موجبة لبكتريا داء المشعرات Chlamydia trichomatis . نتائج فحص تفاعل البوليمر المتسلسل PCR كانت الاكثر دقة في كشف العامل المسبب للامراض المنقولة جنسيا حيث اوضحت 17% من النساء مصابات ببكتريا داء المشعرات.

الكلمات المفتاحية : الامراض المنقولة جنسيا، الاجهاض، تفاعل البوليمر المتسلسل، تقنية الاليزا

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Introduction

Sexually transmitted infections refer to many types of clinical syndromes and infections caused by pathogens that can be acquired and passed by

sexual contact [1]. These infections are caused by more than 30 different bacteria, viruses and parasites, transmitted through person to person by vaginal, anal and oral sex. The most common

infections are Gonorrhoea, Chlamydia infection, syphilis, trichomoniasis, chancroid, genital herpes, genital warts, human immunodeficiency virus (HIV) and hepatitis B infection [2]. Most sexually active people will be infected with a sexually transmitted infection (STI) at some point in their lives [3].

Recurrent Spontaneous Abortion, Habitual Abortion or Habitual Miscarriage is the loss of 3 or more sequent pregnancies before the 24th week of pregnancy [4]. It can be further divided into two types on the basis of number of abortions: Sporadic spontaneous abortion; only one abortion and recurrent spontaneous abortion (referred to as habitual abortion) historically defined as 3 or more consecutive pregnancy losses prior to 20 weeks of gestation from last menstrual cycle [5]. Many sexually transmitted pathogens and their infections are a known cause of fetal loss in and humans [6].

Material and methods

Study groups

The study was carried out on specimens obtained from one hundred female patients attending private gynecology clinics, between the end of November 2017 and the first of February 2018. Their age were between (15-45) years. For each patient a case report was prepared. For each patient Blood samples, mid-stream Urine samples were taken for aborted women that were carefully selected and examined by gynaecologists. The criteria for the case group was the women suffering from abortion from one up to four times, these women were excluded for other causes of abortion other than sexual transmitted infections, such as TORCH.

Specimen Collection

The blood specimen of 5 ml was collected by vein puncture using 5 ml syringe from all patients and control group. The blood was allowed to clot at room temperature, then centrifuged at 5000 rpm for 10 minutes, the sera collected from blood sample by centrifugation were kept in -20 C until used for immunodiagnostic test.

The urine sample of patients collected in sterile urine cups, sample was kept in -20 C freezer for DNA extraction.

Enzyme immunoassay for the detection of IgM antibodies to Chlamydia trichomatis in human serum:

This test was used for the detection of species specific IgM and antibodies to C.trichomatis according to manufacture (demeditec).

DNA extraction:

DNA was extracted from urine sample by using commercial purification (Presto mini Gdna Bacteria kit).

Amplification and detection of Chlamydia traichomatis by multiplex Polymerase chain reaction.

Different annealing temperature was used for optimization of amplification protocol. Conventional PCR was carried out on all DNA samples to amplify a fragment.

Samples cross contamination problems were avoided; following a number of precautions including performing DNA extraction in laminar flow hood with subsequent irradiation by UV light and use of three separated areas for the DNA extraction, preparation of PCR mixture, PCR amplification and running gels.

Specific Primers:

Primers were provided in a lyophilized form and were dissolved in sterile D.W to give a final concentration of 10 pmo\ul as recommended by the provider (Table (1)).

Table 1: Primers Used in the Experiment

Genes	Sequences	Size	Reference
Chlamydia major outer membrane protein	F- GGGAATC CTGCTGAA CCAA	19	Muvuny et al.,2011
	R- TCAAAAC ACGGTCG AAAACA	20	

Statistical Analysis

All data of the present study were analyzed statistically by using IBM SPSS software packaged version (23.0), by employing Chi – square test and significance of the obtained results were judged at the 0.05.

Results

The studied group of women was ranged from 15-45 years. The highest number of aborted women was among the women who were 35 -39 years old (26 %), followed by the age group of (23%), The highest infection number of C. trichomatis IgM was found positive in 7 (7%) of women with history of abortion (Table 2).

Table 2: Frequency of Positive IgM C.trichomatis among women aborted women

	Frequency	Percent
Positive	7	7%
Negative	93	93%
Total	100	100%

Successful amplification of C.trichomatis gene by PCR was considered as a positive result by multiplex conventional PCR. Out of 100 DNA samples extracted from urine samples tested, 17 (17%) were positive for C.trichomatis (Fig 1) & (Table3).

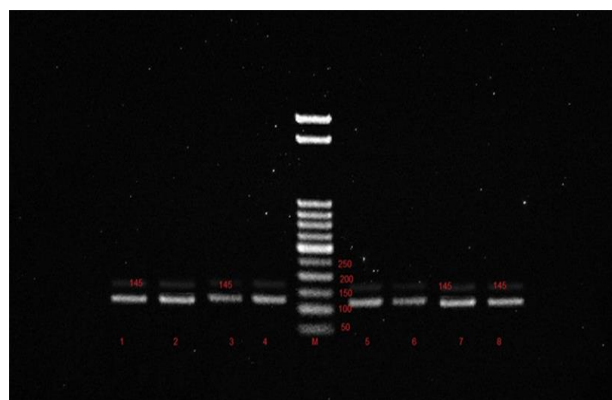


Fig 1: ethidium bromide- stained agarose gel of multiplex PCR amplified products from chlamydia trichomatis major outer membrane protein, agarose 2%, and TBE(1X) at 70 V/cm for 90 min., stained with safe red dye and visualized on a UV transilluminator. Lane M DNA ladder, lane [1-2-3-4-5-6-7-8] urine sample

Table 3: The Frequency of Positive C.trichomatis by Conventional PCR

	Frequency	Percent
Positive	17	17%
Negative	83	83%
Total	100	100%

Discussion

C. trachomatis is one of the most frequent causes of sexually transmitted diseases and could seriously affect public health. Therefore, effective epidemiological control starting with an adequate method for correct and sensitive diagnosis is required [7,8]. The data presented in this study demonstrate, by PCR, up to 17% of the women who attended Al-Kut Hospital for gynaecology and the private clinics suffering from recurrent abortions. Sampling was based on the history of the patients with a number of abortions. The case group was women suffering from abortion during their pregnancy, women who did not complete their pregnancy successfully. The study excluded the patient with other infectious diseases such as toxoplasma, rubella, herpes, or other cases of abortion such as chromosomal abnormalities or hormonal.

Age group was ranging from 15 -45 years. The highest number of aborted women was among the women who were 35 -39 years old (26 %), followed by the age group of (23%), This result was also agreed with a study [9]. which showed the average abortion rate is (27% years old).

The majority of abortions (95.5%) were announced to be spontaneous and 3.9% of the abortions were persuaded by the patient [10]. This disagreement is probably because of the large number of samples included. Regarding the rates for women aged 30-34 have increased steadily in 2007 to 2017, and rates for women aged 35 and over have also in 2007 in 2017 [11]. risk of abortions was significantly increased in women at higher age (>33 years) [12]. serum *Chlamydia trachomatis* IgM were assessed by ELISA. 100 serum sample from aborted women. on comparison between the data obtained for serum anti *chlamydia trachomatis* IgM.

In this study screening of the anti-*chlamydia* antibody by ELISA results of positive (7.0%), negative (93.0%) of the case group (Table 1). The rate of *chlamydia* infection detected by ELISA among Saudi women agreed with the findings of this study [13].

The relationship between *chlamydia trachomatis*–positive serologic results and abortion remained significant after adjustment for age, origin, level of education, and number of sex partners; this disagreement is probably because of the different criteria of the study group and the sample size [14]. In Baghdad risk group for sexual transmitted diseases were studied for prevalence of anti-*Chlamydia* by using ELISA technique.

Results indicated that prevalence of *C. trachomatis* was 66% among normal population High prevalence of low levels were detected among normal population 45% compared to group 33% which was non-significant [15]. This disagreement might be because of the different case group and the difference of method of sample collection.

The intracellular life style of *chlamydia* and the capability to cause constant infections with low-grade replication order tests with high analytical sensitivity to instantly detect *C. trachomatis* in medical samples. Nucleic acid amplification tests are the most sensitive assays with a specificity similar to cell culture and are considered the method of choice for detection [16]. When diagnostic methods are faster and results more accurate, they are restricted to improve patient care. As the use of automated and standardized methods increase and human error decreases, more laboratories will embrace molecular testing methods [17]. PCR was positive for *chlamydia trachomatis* infection in women [18]; this is relatively close to the current study.

Conclusions:

Nucleic acid amplification test by using Polymerase chain reaction proved to be superior and more efficient in the diagnosis of Chlamydia trachomatis than Enzyme linked immune sorbent assay.

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