Full Length Research Paper

Molecular detection of *Chlamydia trachomatis* among gynecological patients attending Khartoum Teaching Hospital

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To perform moleular detection of *Chlamydia trachomatis* among gynecological patients attending Khartoum Teaching Hospital. 200 endocerical smears were collected randomly from out-patient infertile women attending the Department of Obsetrcs and Gyencology, Khartoum Teaching Hospital (Sudan). Detection of *C. trachomatis* was performed by the polymerase chain reaction (PCR) technique. About 191 out of 200 patients having vaginal discharge were investigated, 43 (22.5%) of them were found positive for *C. trachomatis* by PCR test. Most positive cases were in the age range 26-40 years, whereas negative cases were in the age range 61 years and above. The age range (20-40) years, is a risk factor that exposes sexually-active women to *C. trachomatis* infection. Also, *C. trachomatis* infection in pregnant women may be associated with abortion and pelvic inflammatory disease. *C. trachomatis* infection is becoming a public health problem in Khartoum due to its highest frequency among infertile Sudanese women.

Key words: *Chlamydia trachomatis*, polymerase chain reaction, Sudanese women.

INTRODUCTION

Chlamydia trachomatis is one of the most common sexually transmitted bacterial infection in the world and sexually active young persons are at highest risk. The inciedence of chlamydia infection in women with tuboperitareal sterility is approximatley 80%. Clinically inflammotary disease of the pelvic organs in such an infection is characterised by primary chronic course and involvement of the cervix (Akush, 1993). *C. trachomatis* is the most common bacterial sexually transmitted infection (STI) in many developed countries. The highest prevalence rates are found among young adults who have frequent partner change rates (Christian et al., 2012).

In Sudan there is no recent published data about the prevalence of *C. trachomatis* infection among infertile and pregnant women, however, there is highly sensitive and specific techniques have been developed for diagnosos of *C. trachomatis*. *C. trachomatis* infection have been reported to cause silent infections (asymptomatic) in communities which becomes endomic and could remain unnoticed for very long time. In most parts of Sudan these organisms are not screened for, and hence relative infermation about frequencies of the organisims are sparse (Okoror et al., 2007). The aim of this study is to perform moleular detection of *C. trachomatis* among gynecological patients attending Khartoum Teaching Hospital.

MATERIALS AND METHODS

This is a facility based qualitative study to determine the prevalence

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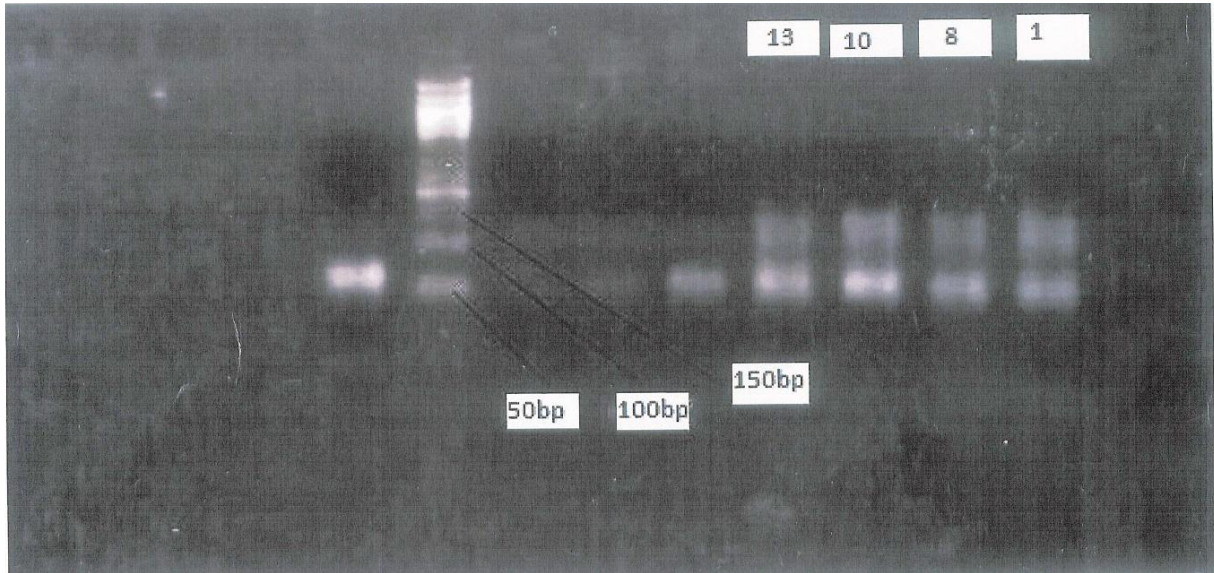*Corresponding author. E-mail: msud22@hotmail.com. Tel: 00249123632116.

Figure 1. *C. trachomatis* PCR result.

of *Chlamydia* infection among Sudanese women. It is a descriptive cross-sectional study to determine the frequency of this infection. The study was carried out in two hundred women, attending Khartoum Maternity Hospital (Sudan).

DNA extraction

Endocevical samples were collected in 1 ml lysis buffer containing 5 µl proteinase K, 500 µl guanidine chloride, and 150 µl NH₄ acetate. All were mixed and incubated at 37°C overnight. The mixture was boiled, cooled to room temperature, and then 2 ml pre-chilled chloroform were added. It was then vortexed and centrifuged for 5 min at 3000 rpm. The upper layer was transferred to another tube and 6 ml of cold absolute ethanol were added, shaken, and kept at -20°C for at least 2 h. After that it was centrifuged at 3000 rpm for15-20 min, the supernatant was carefully drained, and the tube was inverted on a tissue paper for 5 min. Then the pellet was washed with 4 ml 70% ethanol. Another centrifugation at 3000 rpm for 15 min was made, and the supernatant was poured off and the pellet was allowed to dry for 10 min. Later the pellet was re-suspended in 200 µl deionized water, briefly vortexed and kept at 40°C overnight. Lastly the DNA extract was aliquoted as a stock solution and stored at –20°C (Fallah et al., 2005).

C. trachomatis primers such as CT1: (CCT/GTG/GGG/AAT/GCT/GCT/GAA) and CT4: (GTC/GAA/AAC/AAA/GTCATCCAGTA/GTA were drived from highly conserved regions of the published DNA sequences for *C. trachomatis* species were used in this study.

Polymerase chain reaction (PCR) analysis

Two microliter of DNA extracts were processed in a 30 µl reaction volume containing PCR buffer (10 Mm Tris , 50 Mm KCL , 0.01% gelatin 200 µM deoxynucleoside triphosphate, 2.5 mM MgCl₂, 0.5 µM of each primer, and 1 U of tag polymerase. The first cycle, consisting of 5 min denaturation at 94°C, followed by 35 cycles each of 30 s at 94°C, 45 s at 56°C and 1 min at 72°C with a final extension for 10 min at 72°C. The PCR products were visualized in 2% agarose gels containing 0.5 µg of ethidium bromide/ml. The

edges of the product were sealed carefully, using sealing tape, and then 1.5% agarose gel was prepared by adding 54 ml DW, 6 ml 10x tris base boric acid EDTA, 90 µm tris borate, and 2 µm EDTA (pH 8.0) to 0.9 g agarose gel. The mixture was melted in a boiling water bath, then it was cooled to 60°C, and 3 µm of ethidium bromide (10 mg/ml) were added to the gel, and mixed thoroughly. The mixture was then poured into horizontal electrophoresis gel tank with suitable size combs, and the gel was left for 30-45 min to polymerize. Running buffer was added containing 250 ml distilled water and 15 ml buffer to cover the gel. 7 µl of PCR product was located into the comb wells, and 5 µl of DNA marker were also located. The run was performed at 100 Volt, and current range was 3-8 ml Ampere for 30 min. The gel was visualized over ultraviolet trans-illuminator and photographed using gel documentation system. Fragements size was estimated from the distance of migration velative to the positive control (Fallah et al., 2005). We used positive control of *C. trachomatis* with length of 50 bp to determined the size of the amplified DNA.

RESULTS

A total of 200 endocerical smears were collected randomly from out-patient infertile women attending the Department of Obstetrics and Gyencology (Khartoum Teaching Hospital). The endocervical smears were tested for *C. trachomatis* using PCR technique (Figure 1). From the 200 patients investigated, 191 were presenting with vaginal discharge, and 43 (51.2%) of them were found positive for *C. trachomatis* by PCR technique. Most positive cases were in the age range 26- 40 years, where as negative cases were aged 61 years and above (Table 1).

Nine patients without vaginal discharge were investigated for *C. trachomatis*, 5 of them (55. 5%) were found positive for *C. trachomatis* by PCR technique (Table 2).

Table 1. Detection of *Chlamydia trachomatis* among infertile vaginal discharge women according to age incidence.

Age range (years)	Positive PCR (%)	Negative PCR (%)	Total
15-25	9 (45)	11 (55)	20
26-40	30 (50.8)	29 (49.2)	59
41-60	4 (80)	1 (20)	5
61 and above	0 (0)	0 (0)	0
Total	43 (51.2)	41(48.8)	84

Table 2. Detection of *Chlamydia trachomatis* among infertile women without vaginal discharge according to age incidence.

Age range (years)	Positive PCR (%)	Negative PCR (%)	Total
15-25	1 (10)	9(90)	10
26-40	3 (50)	3(50)	6
41-60	1 (10)	9 (90)	10
61 and above	0 (0)	20 (100)	20
Total	5 (10.5)	41(89.5)	46

Table 3. Detection of *Chlamydia trachomatis* infection among infertile PID patients according to age incidence.

Age range (years)	Positive PCR (%)	Negative PCR (%)	Total
15-25	8 (53.3)	7(46.7)	15
26-40	23 (47.9)	25(52.1)	48
41-60	4 (40)	6(60)	10
61 and above	0 (0)	23(100)	23
Total	35 (36.4)	61(63.6)	96

Table 4. Detection of *Chlamydia trachomatis* among infertile women according to risk factors.

Risk factor	Positive PCR (%)	Negative PCR (%)	Total
Low education	19 (44.2)	24(55.8)	43
Smoking	15 (44.1)	19(55.9)	34
Promosecuity	26 (52)	24(48)	50
Total	60 (47.2)	67(52.8)	127

73 pelvic inflammatory disease patients (Known case which were already diagnosed by labroscopy), out of the 200 infertile patients were investigated for *C. trachomatis*. 35 (47.9%) of them were positive by PCR technique. The frequency of PID was higher in the age range 26-40 years (Table 3). 60 (47.2%) of PCR positive women were found exposed to *Chlamydia* infection risk factors, such as smoking, low education, and promosecuity (Table 4). We found that there are association with *C. trachomatis* and smoking, then those people have highly sexualty active behavior.

On the other hand, *C. trachomatis* was detected among 11 cases (36.0%) with PID, 13 cases (41%) with vaginal discharge, 10 cases (38%) with past history of abortion, 12 cases (41%) with PID and vaginal discharge, and 9 cases (56.2%) with PID, vaginal discharge, and abortion.

DISCUSSION

Screening of *C. trachomatis* is needed to define measures of prevention, modes of transmission to newborn, and ways to reduce sexual spread. *C. trachomatis* is most comon agents leading to congenital infection in both men and women. Worldwide the estimated annual incidence goes up to 50 million cases of *C. trachomatis*

infection. *C. trachomatis* in women has a clinical course varying from asymptomatic infections to ascending infections leading to pelvic inflamatory disease asso-ciated with late ectopic pregnancy and tubal infertility (Land, 2010).

A total of 191 patiens with vaginal discharge were investigated, 43 (51.2%) of them were found positive for *C. trachomatis* by PCR tehnique. This indicates that the molecular detection of *C. trachomatis* by PCR is a useful technique. Most positive cases were in the age range of 26- 40 years, where as negative cases were aged 61 years and above (Table 1).

In this study 43 (51.2%) patients were positive by PCR technique. This result was higher than results of a previous study performed by Ortashi et al. (2004) who found a prevalence of 7.3% of *Chlamydia* infection among Sudanese women attending an obestetric and gynecology clinic in Kartoum. Also in the present context, the frequency rate of *Chlamydia* infection among women investigated was 51.2% which was higher than the frequency rate of *Chlamydia* infection among men (10.4%) that was reported by Omer et al. (1985).

In this study 17% of the fertile pregnant women were found positive for *C. trachomatis*. This figure was similar to that reported (16.4%) by Nafi (2006) study in Khartoum (Sudan), where he found a frequency of 16.4% *C. trachomatis* infection among pregnant fertile women. This author also reported a frequency of 43.4% among women with past history of abortion and 52% among PID patients. In this study *C. trachomatis* infection detected was (38%) among women with past history of abortion and (56.2%) among PID patient .

In the present context *C. trachomatis* was detected in 43 patients (21.5%) using PCR tchnique. This result is higher than that reported by Fallah et al. (2005) who detected *C. trachomatis* in 14 patients (14.9%).

Land (2010) in Netherlands studied asymtomatic cervical *C. trachomatis* infections in association with age incidence using PCR technique. They found the overall prevalance rates of *C. trachomatis* infection were 9.2 and 11.8% among patients youger than 30 years and above 30 years respectively. While in this study the frequency rates of *C. trachomatis* detected by PCR were 69.8% in the age range 26-40, 21% in the age range below 26 years, 9.3% in the age range above 41 years. This result reflects a significant association between the age range 26-40 years and *C. trachomatis* infection.

In conclusion, the age range (20-40) years (Table 3) is a risk factor that exposes sexually-active women to *C. trachomatis* infection. Also *C. trachomatis* infection in pregnant women may be associated with abortion and pelvic inflammatory disease. *C. trachomatis* infection is becoming a public health problem in Khartoum due to its highest frequency among fertile and non-fertile Sudanese women.

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