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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet

Asian Network for Scientific Information

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (24): 4490-4494, 2007
ISSN 1028-8880
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Chlamydia trachomatis Prevalence in Iranian Women Attending Obstetrics and Gynaecology Clinics

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Abstract: This study was designed to estimate the prevalence of *Chlamydia* infection in women attending Obstetrics and Gynaecology clinics in Tehran, during May 2003 to October 2003. Women attending Obstetrics and Gynaecology clinics aged 15-42 were recruited by Sequential Random Sampling. Those who had not passed urine in the last hour were eligible. Informed consent was obtained and a questionnaire completed after being interviewed by a midwife. First void urine was collected and after DNA extraction from urine specimen, PCR tests were performed; urine DNA samples were tested by strand displacement amplification (SDA) for *Chlamydia* confirmation. 12.6% (133/1052) tested positive for *Chlamydia* by PCR. Of these PCR positive samples, 86 were available for re-testing by SDA and 67 were positive giving a correlation between the tests of 78%. This gave an overall true prevalence of 6.4% which is however, underestimated. No statistical differences were seen between patient age groups, details of personal and reproductive history and combined PCR and SDA positivity for *C. trachomatis*. A 12.6% prevalence of *Chlamydia trachomatis* was found by PCR testing which is cost effective to screen and treat. Despite limitations in re-testing PCR-positive samples by SDA, a 78% correlation between tests confirms a high prevalence of *C. trachomatis*. Non-invasive screening of women was therefore a success in this group of patients. As this was the first time that more sensitive molecular methods were used for detection of *C. trachomatis*, prevalence in such a big sample size, the results are considerable. However, we suggest further such testing.

Key words: *C. trachomatis*, prevalence, Iran, women, PCR

INTRODUCTION

Although it is widely known that *Chlamydia trachomatis* is the most prevalent bacterial cause of sexually transmitted infections worldwide (Paavonen and Eggert-Kruse, 1999), little information is available on its prevalence in the Middle East. Two recent studies in the United Arab Emirates and Jordan have shown prevalence rates of 3 and 5%, respectively (Ghazal-Aswad *et al*., 2004; Awwad *et al*., 2003). To the best of our knowledge there have been only few published reports on prevalence of genital chlamydial infections in females in Iran. Of these, one reported 15.5% prevalence in women with cervicitis (Zaieimi *et al*., 2006) and one of the oldest studies in Iran reported a 7% isolation rate in prostitutes (Darougar *et al*., 1983). Other studies of relevance were in Farsi (abstract in English) and reported prevalence rates of 7 and 3%, respectively using

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Direct Immunofluorescence (Mir Mahdavi *et al.*, 1998; Behroozi and Badami, 1999). Recently a study has been reported using a Nucleic Acid Amplification Test (NAAT) which gave a prevalence of ≥14.9%. This used MOMP PCR to detect *C. trachomatis* in urine of women with cervicitis (Fallah *et al.*, 2005). No known studies have been reported including such an extended sample size from areas within the city of Tehran.

A positive diagnosis of *C. trachomatis* is especially useful in asymptomatic women, where treatment has been shown to reduce complications such as Pelvic Inflammatory Disease (PID), ectopic pregnancy and tubal factor infertility (Scholes *et al.*, 1996; Kamwendo *et al.*, 1996). However, cost effective analyses demonstrate that screening with NAATs and treating women with *C. trachomatis* in the community only becomes worthwhile if the prevalence exceeds 4% (Genc and Mardh, 1996; Paavonen *et al.*, 1998). Therefore, some previous Middle Eastern studies would suggest that such a screening programme might not be particularly cost effective.

The present study was conducted in Tehran, the capital city of Iran as part of a WHO funded epidemiological survey of *C. trachomatis* using a PCR based method on first-void urine; SDA was used as a confirmatory test on extracted DNA. We describe the prevalence, personal history and reproductive history in a female population.

MATERIALS AND METHODS

Study cohort: A population of 1052 women attending five Obstetric and Gynaecology clinics from different parts of Tehran between May 2003 and October 2003 was selected by Random Sampling. Women aged 15-42 and who had not passed urine in the past hour was eligible for inclusion.

Personal and reproductive histories were obtained and recorded in a questionnaire after being interviewed by a midwife. Informed consent was obtained and a first void urine specimen was collected. Questionnaires, consent forms and specimen containers were coded and retained.

Diagnostics: Urine (10-50 mL) was brought from the clinics to the Avesina Research Institute in Tehran and DNA extractions made the same day on the urine deposit by the method of Sambrook and Russell (Sambrook and Russell, 2001). Extracted DNA samples were kept at -70°C until analysed. *C. trachomatis* plasmid DNA was amplified using validated and published primers (Claas *et al.*, 1990) Chl-S: GGA CAA ATC GTA TCT CGG and Chl-AS: GAA ACC AAC TCT ACG CTG which gave a PCR product of 517 bp. This in house PCR method first underwent routine validation testing (Fig. 1) and then applied to clinical samples (Fig. 2).

Eighty six DNA samples positive for *C. trachomatis* by PCR were transported on ice to the UK where they were tested blind by SDA (Becton Dickinson, Cowley, UK). Eighty PCR negative DNA samples were tested by

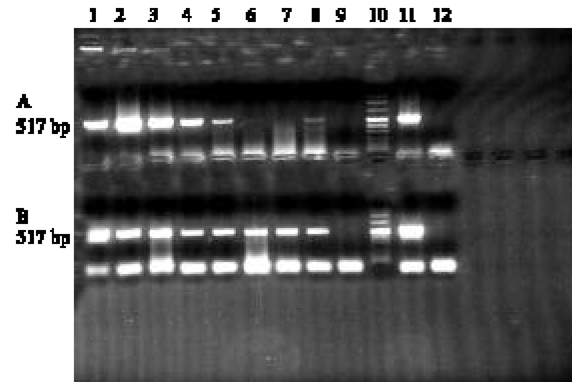

Fig. 1: *C. trachomatis* PCR amplification (40 cycles) of the washed precipitates of a standard strain of the bacteria diluted in a urine sample. The precipitates were prepared either immediately after preparation of the 10 fold dilution series (Panel B) or a few hours later (Panel A). Lanes 1 to 8 show PCR amplifications of the different dilutions from 10^1 to 10^8, respectively. Lane 9 shows PCR product of the uncontaminated urine. Lane 10 shows DNA molecular weight standard VIII (Roche). Lanes 11 and 12 show the positive (bacteria alone) and negative (water) controls, respectively

Fig. 2: Agarose gel electrophoresis of ct specific PCR and relevant RFLP on patients urine sample. Lane 1 and 2 show the negative control and its related RFLP respectively. Lane 3 and 4 show the PCR product and RFLP for the positive control. Lane 5 shows 100 bp size markers. Lane 6, 8, 10, 12, 14 and 16 show PCR products and lane 7, 9, 11, 13, 15 and 17 show the corresponding RFLP for the patients urine samples

SDA at the same time. The SDA test was performed as per the manufacturer's instructions except that the urine deposit was substituted with approximately 100 μL of DNA sample. Specimens were considered true for *C. trachomatis* if they were positive by both NAATs.

Statistical analysis: Statistical data were analysed by using SPSS package version 11. Means for risk factors were compared using the student t-test and analysis of variance (ANOVA) to determine statistical significance.

Ethics approval: Ethical approvals were received from the Avesina Research Institute ethics committee.

RESULTS

Within the cohort, details of 1052 patients were studied although some questionnaires were incomplete. PCR was positive in 133 (12.6%) and the prevalence of true positives (PCR positive, SDA positive) was 6.4% (95% CI: 4.9-7.9) (67/1052) However, as a significant number of PCR positive samples were unavailable for testing by SDA, the true prevalence rate is likely to be much higher. All PCR negative DNA samples except for one, tested by SDA were negative.

Participants were 15-42 years old (medium 28.52±6.36). Although if seemed that infection was more prevalent among participants aged more than 30 years old.

When details of true positive samples among the three age groups were examined, interestingly there were no significant differences (Table 1).

Table 1: The positive rate of *C. trachomatis* in different age groups

Patient age group in years (n = No. of patients)	*C. trachomatis* PCR and SDA positive prevalence rate (%)
<20 (n = 93)	4.3
20-30 (n = 544)	6.3
>30 (n = 399)	7.3
Total = 1036	Mean = 6.4

Table 2: Personal history and combined PCR/SDA prevalence of *Chlamydia trachomatis*

History		No./(%)	Positive test	Prevalence (%)	p-value†
Employed	Yes	84 (8.2)	9	10.7	0.080
	No	935 (91.8)	54	5.8	
Married	Yes	991 (94.2)	64	6.5	0.450
	No	61 (5.8)	3	4.9	
Sexual activity	Yes	985 (93.8)	63	6.4	0.404
	No	65 (6.2)	3	4.6	
Contraception	Yes	502 (48.1)	32	6.4	0.474
	No	542 (51.9)	33	6.1	
Pregnant	Yes	340 (32.5)	25	7.4	0.201
	No	707 (67.5)	41	5.8	
Smoker	Yes	17 (1.6)	2	11.8	0.290
	No	1034 (98.4)	64	6.2	
Drug addiction	Yes	2 (0.2)	0	0.0	0.878
	No	1049 (99.8)	66	6.3	

†: A p-value of ≤0.05 is significant

Table 3: Reproductive history and combined PCR/SDA prevalence of *Chlamydia trachomatis*

History		No./(%)	Positive test	Prevalence (%)	p-value†
Vaginal discharge	Yes	409 (39.0)	29	7.1	0.234
	No	640 (61.0)	37	5.8	
Pelvic pain	Yes	135 (12.9)	8	5.9	0.849
	No	913 (87.1)	58	6.4	
Ectopic pregnancy	Yes	10 (1.0)	1	10.0	0.479
	No	1039 (99.0)	65	6.3	
Spontaneous Abortion	Yes	222 (21.2)	20	9.0	0.060
	No	827 (78.8)	46	5.6	
PROM	Yes	68 (6.5)	3	4.4	0.366
	No	981 (93.5)	63	6.4	
Low birth weight infant	Yes	28 (2.7)	2	7.1	0.536
	No	1021 (97.3)	64	6.3	
IVF history	Yes	76 (7.2)	6	7.9	0.342
	No	973 (92.8)	60	6.2	

†: A p-value of ≤0.05 is significant

93.8% of participants were sexually active and 91.8% were married. Based on combined PCR/SDA results the infection was more prevalent among participants with past history of ectopic pregnancy (10%), spontaneous abortion (9.0%), IVF history (7.9) and vaginal discharge (7.1%); But when both personal and reproductive histories (Table 2, 3) respectively of all patients were examined for associations of positivity for *C. trachomatis*, no statistical differences were found. However, those patients with a history of spontaneous abortion (Table 3) were more likely to be positive for *C. trachomatis*.

DISCUSSION

This is to the best of our knowledge, the first time that such a large survey has been undertaken of women for *C. trachomatis* prevalence using NAATs in Iran. Using PCR testing on first-void urine, a prevalence rate of 12.6% was obtained. As only 86 PCR positive samples were available for SDA testing, a finding of 67 SDA positive samples gave a correlation of 78% and a true prevalence rate of 6.4%. We appreciate that an 78% correlation rate between PCR and SDA is somewhat disappointing. However, it should be stressed that very little DNA sample remained in several cases, the samples had been stored for a considerable period of time and they then had to undergo transportation from Tehran to Sheffield. These results do suggest that the true prevalence rate of 6.4% which we quote is significantly underestimated. Moreover, the fact that we discovered one sample positive by SDA and negative by PCR out of a total of 80 PCR negative samples is not particularly surprising when methods are compared and may simply reflect the presence of a urine inhibitor which has been removed on freezer storage.

In comparison with other studies in Middle Eastern countries, surprisingly, the prevalence rate seems high (Ghazal-Aswad *et al*., 2004; Awwad *et al*., 2003). However,

it should be realised that as there are no STI clinics in Iran, some women attending obstetrics and gynaecology clinics might otherwise have gone elsewhere for consultation in other countries. Potentially, therefore, this could positively bias the findings. Nevertheless, our findings confirm those of Fallah *et al.* (2005), who also found a high prevalence especially in the 28 to 38 year old patient group. Although figures of 12% and higher have been reported for *C. trachomatis* prevalence in asymptomatic women in Europe (Wilson *et al.*, 2002), typically females would belong to a younger mean age group than those in this study. However, recent studies in Belgium and Finland have shown that the prevalence of *C. trachomatis* in those aged 25-29 years did not decline in comparison with younger age groups and these findings may be important in this study as that was the predominant age group (Verhoeven *et al.*, 2003; Paukku *et al.*, 2003). This is important because screening programmes traditionally focus on women under 25 (Pimenta *et al.*, 2000).

Interestingly, we found a strong association between a history of spontaneous abortion and an increased prevalence of *C. trachomatis* in this patient group. This finding is similar to those reported by others and suggests that spontaneous abortion may be an important risk factor for chlamydial infection (Lunenfeld *et al.*, 1989; Witkin *et al.*, 1995; Witkin and Ledger, 1992; Quinn *et al.*, 1987).

Limitations: An important limitation of our study was the inability to do testing for *C. trachomatis* on urine samples using commercial molecular techniques; they are currently unavailable in Iran.

This meant that only an in-house PCR test could be done, with SDA performed in the UK on DNA extracted from a proportion of positive and negative samples for confirmation.

Another limitation was the possibility that any inhibitors in urine samples, which were not investigated in this study, could also have resulted in false negative PCR findings again raising the possibility of a higher true prevalence rate.

Only one of PCR negative samples was positive by SDA which confirms that DNA extraction was largely successful and the samples were mostly free of inhibitors.

Cost effectiveness: As screening for *C. trachomatis* is cost effective at the high prevalence rate seen in this study, it is imperative that similar prevalence studies are performed in Iran. If high prevalence rates are confirmed, then screening and treating women attending Obstetrics and Gynaecology clinics in Iran would be highly appropriate and should be introduced with some urgency.

CONCLUSION

A true prevalence rate for *C. trachomatis* of 6.4% was found in 15-42 year old women attending Obstetric and Gynaecology clinics in Iran although this figure was an underestimate. The only risk factor of note, although not statistically significant, was a history of spontaneous abortion. Non-invasive screening of women was shown to be feasible in the population selected. It is important that further studies be carried out with some urgency so that a screening programme be implemented to reduce the burden of chlamydial disease.

ACKNOWLEDGMENTS

The authors would like to thank WHO/EMRO (grant No. R6/18/3, ID RPC 02/86) for providing some of the resources for the study, to colleagues in the Avesina Research Institute and the Department of Microbiology, Northern General Hospital, Sheffield for performing the SDA tests.

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