CASE-CONTROL STUDY OF ECTOPIC PREGNANCIES IN MYANMAR: INFECTIOUS ETIOLOGICAL FACTORS

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Abstract. We studied the role of infections in ectopic pregnancy and the different methods of detecting *Chlamydia trachomatis* infection using serology, cervical and tubal PCR assays, by using a hospital-based, case-control study conducted between November 2007 and September 2009. The sample size was 339 with 113 cases and 226 controls. The cases were women admitted for the management of ectopic pregnancy while the controls were women admitted for spontaneous miscarriage. Both cases and controls were tested for syphilis and chlamydial infection by serology. In addition, cervical samples from controls and both cervical and tubal samples from cases were examined for the presence of chlamydia and gonococcal DNA. Sociodemographic data and past histories were collected using set Proforma. Independent variables for multivariate analysis included previous history of spontaneous abortion, ectopic pregnancy, symptoms of sexully transmitted infections (STI), and use of contraception. Women with a previous history of ectopic pregnancy (adjusted OR 28.3; 95% CI 5.8-138.8; p=0.01) and a past history of having had symptoms of STI (adjusted OR 11.06; 95% CI 5.45-22.44; p=0.0005) were significantly more likely to have an ectopic pregnancy than those without such a history. Syphilis serology was positive in 13.3% of ectopic pregnancy cases compared to only 3.5% of controls (crude OR 0.24; 95% CI -0.10-0.58; p=0.001). From cervical swabs, chlamydia DNA was detected significantly more frequently in cases than controls (8.0% *vs* 2.2%; crude OR 0.261; 95% CI -0.09-0.80, p=0.012) but gonorrhea DNA detection rates were not significantly different (3.5% *vs* 0.9%, crude OR 0.24; 95% CI -0.04-1.35; p=0.1). Chlamydia was positive in cases only as diagnosed tubal samples for PCR in 17 (15.0%), cervical samples for PCR in 9 (8.0%) and IgM ELISA in 6 (5.3%). Among the three STI tested for in this study, *C. trachomatis* was the most frequently associated with ectopic pregnancy and was more frequently diagnosed by PCR on tubal samples than PCR on cervical samples or chlamydia IgM serology.

Keywords: ectopic pregnancy, sexually transmitted infections, case-control study

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INTRODUCTION

Sexually transmitted infections (STIs) are a serious health problem in developing countries. In Myanmar, they have been ranked as the 14 of 39 in priority

of health problems (Ministry of Health, 2008). Ectopic pregnancy following scarring of the fallopian tubes is a serious sequela of STI. In Myanmar, the prevalence of ectopic pregnancy has increased over the past two decades (Sandar-Win, 1998; North Okkatapa General Hospital, 2005) and several epidemiological studies have been carried out to determine associated factors, such as an adverse obstetric history and gynecological complications (Zarni-Win, 2008). However, none of these studies examined the role of infections on ectopic pregnancies. Hence, this study was undertaken to determine the prevalence of three common STI (genital chlamydia, genital gonorrhea and syphilis) among women presenting with an ectopic pregnancy and comparable controls. Chlamydia and gonorrhea salpingitis are known predisposing conditions for ectopic pregnancy. Syphilis was included as a marker of sexual activity. Different methods for detecting *C.trachomatis* were also assessed for their relative sensitivity in the diagnosis of upper genital tract infection.

MATERIALS AND METHODS

A hospital-based, comparative, case-control study was conducted between November 2007 and September 2009 in Thingangyun Sanpya and North Okkalapa General Hospitals in Yangon, Myanmar. The study population consisted of women 18 to 45 years old, who were admitted to hospital because of ectopic pregnancy, and controls of the same age who were admitted to the hospital for first trimester spontaneous miscarriage leading to incomplete abortion. A sample size of 339 (113 cases and 226 controls) was determined by the method of Schlesselman (1982). Women who refused to give consent for participation, cases who were found not to have tubal ectopic pregnancy at the time of surgery and controls who presented with miscarriage following an induced abortion were excluded. Informed consent was obtained from all study participants and the study was approved by the Ethics Committee, Department of Medical Research (Lower Myanmar).

Specimen collection

Blood was collected for syphilis and chlamydia serology, and cervical swabs were obtained for chlamydia and gonorrhea PCR from each participant. Tubal samples were collected from ectopic cases at the time of laparotomy for chlamydia and gonorrhea PCR. All tubal and cervical specimens were transported to the Bacteriology Research Division, Department of Medical Research (Lower Myanmar) in tubes of normal saline kept in ice. These samples and sera were kept frozen at -20°C until testing.

Collection of data using set Proforma

After obtaining informed consent, study participants were interviewed to obtain sociodemographic, reproductive, contraceptive usage, gynecological, medical and surgical history. The interviews were conducted the day after surgery, in a room where privacy was ensured. Data were collected according to set Proforma and confidentiality of records was ensured using identity codes and keeping records in a locked cupboard with limited access.

Laboratory procedures

Syphilis screening was carried out using the Rapid Plasma Reagin (RPR) (Immutrep) and Treponema Pallidum HaemAgglutination (TPHA) tests. Only results positive on both the RPR and TPHA tests were used for analysis. Chlamydia IgM ELISA testing was carried out (TayTec, Canada) according to the manu-

Table 1
Primers and amplification parameters used for Multiplex PCR.

Target	Primer sequence	Reference
Chlamydia trachomatis	KL_1 5′tcc-gga-gcg-agt-tac-gaa-ga 3′ KL_2 5′aat-caa-tgc-ccg-gga-ttg-gt 3′	Mahony *et al*, 1992
Neisseria gonorrhoeae	HO_1 5′ gct-acg-ca_t-acc-cgc-gtt-gc 3′ HO_3 5′cga-aga-cct-tcg-agc-aga-ca 3′	Ho *et al*, 1992
Amplification thermal profile	95°C x 5 minutes 1 cycle 95°C x 1 minute] 57°C x 1 minute] 40 cycles 72°C x 2 minutes] 72°C x 7 minutes (Final extension)	

facturer's instructions. Chlamydia and gonorrhea were tested for on cervical and tubal samples using a duplex PCR method as described previously (Nasution *et al*, 2007). For tubal tissue, DNA was extracted after low-speed sonication. The primers and amplification parameters used for the PCR are shown in Table 1. PCR products were visualized on 2% agarose gel subjected to electrophoresis of 100 volts for 30 minutes, followed by staining with ethidium bromide. For each PCR test, positive (*C. trachomatis* and *N. gonorrhoeae*) and negative (reaction mixture without template) controls were included as well as a Lambda phage: Neisseria hybrid DNA internal control (Nasution *et al*, 2007).

Data analysis

Model selection and model fitting. Independent variables for multivariate analysis included previous history of spontaneous or induced abortion, ectopic pregnancy, surgical operations of the lower abdomen, prolonged infertility, tubal surgery, symptoms of STI and use of contraception. The dependent variable was "presence of ectopic pregnancy".

Stepwise logistic regression (forward likelihood ratio method) was used to ascertain possible predictors for the occurrence of ectopic pregnancy, when controlling for other variables. Independant variables included in the model were selected from an initial bivariate analysis with a cut-off point of probability of ≤0.10 for entry and ≥0.20 for removal. Each category was contrasted with the reference category to find the chance of occurrence of ectopic pregnancy.

The Wald test for the chi-square (*Z* statistic) was used to evaluate the relationship between covariates and outcome with a cut-off point $p \leq 0.10$ ($0.05 < p < 0.10$) termed "of marginal significance". There was a difference of -2 log likelihood ratio statistics from the initial model (in step 1) and the final model (step 4) (299.367 *vs* 225.549) (Norusis, 2004).

The Hosmer-Lameshow statistic indicated a good fit for the binary logistic regression model at a significance of >0.05 (p=0.488) which fitted the data adequately (Hosmer and Lameshow, 2000).

RESULTS

Age, marital status, socioeconomic status and parity were not significantly

Table 2
Ectopic pregnancy and past surgical, gynecological and obstetric history.

Variables	Cases *N* (%)	Controls *N* (%)	Crude OR	95% CI	*p*-value
Prior tubal surgery	18 (18.4)	2 (1.0)	0.43	0.01-0.191	0.0005
Prior ectopic pregnancy	28 (28.6)	2 (1.0)	0.02	0.01-0.11	0.0005
Prior spontaneous abortion	9 (9.2)	46 (22.2)	2.83	1.32-6.04	0.006
Prior induced abortion	5 (5.1)	2 (1.0)	0.18	0.04-0.95	0.037
Prior surgical operation in lower abdomen	30 (30.6)	6 (2.9)	0.68	0.27-0.17	0.0005
History of infertility	16 (16.3)	10 (4.8)	0.26	0.11-0.60	0.001

N, Number; OR, Odds ratio; CI, Confidence interval

Table 3
Ectopic pregnancy and contraceptive history.

Variables	Cases *N* (%)	Controls *N* (%)	Crude OR	95% CI	*p*-value
1 pill/month	3 (5.2)	12 (6.3)	0.8	0.2-3	0.762
Monthly injection	1 (1.7)	52 (27.1)	0.047	0.006-0.350	0.000
Condom use	14 (24.1)	5 (2.6)	12	4.1-35	0.000
Vasectomy	7 (12.1)	4 (2.1)	6.5	1.8-23	0.001
1 pill/night	7 (12.1)	46 (24.0)	0.436	0.2-1	0.052
3 monthly injection	26 (44.8)	60 (31.3)	1.8	0.9-3.2	0.056

N, number; OR, Odds ratios; CI, Confidence interval

associated with ectopic pregnancy. Since only two participants gave a history of smoking, this variable was not included for analysis. Almost all participants (96.9% of cases and 93.2% of controls) gave a history of having only one sexual partner. Other variables positively and negatively associated with ectopic pregnancy are listed in Tables 2, 3 and 4 but after the adjusted OR analysis, only previous history of ectopic pregnancy (OR 28.3; p=0005) and past symptoms of STI (OR 11.06; p=0.0005) were found to be significantly associated with ectopic pregnancy (Table 5).

Chlamydia and gonorrhea DNA were detected on cervical swabs in 8.0%, and 3.5% of ectopic cases and in 2.2% and 0.9% of controls, respectively. Syphilis serology was positive in 13.3% of cases and 3.5% of controls. The difference between cases and controls was significant for chlamydia (crude OR 0.261; 95% CI-0.09-0.80; p=0.012) and syphilis (crude OR 0.24; 95% CI-0.10-0.58; p=0.001), but not for gonorrhea (crude OR 0.24; 95% CI-0.04-1.35; p=0.1). Only one case had multiple infections (chlamydia, gonorrhea and syphilis). There was a significant correlation between a positive test for any

Table 4
Ectopic pregnancy and sexual and infectious disease history.

Variables	Cases *N* (%)	Controls *N* (%)	Crude OR	95% CI	*p*-value
History of mucopurulent discharge	47 (48.0)	27 (13.0)	6.1	3.5-11	0.000
History of leukorrhea with pruritus vulvae	32 (32.7)	22 (10.6)	4.1	2.2-7.5	0.000
History of pain in suprapubic area	28 (28.6)	9 (4.3)	8.8	3.9-19.6	0.000
History of ulcers on vulvae	5 (5.1)	4 (1.9)	2.7	0.7-10.4	0.153
History of warts on vulvae	24 (24.5)	14 (6.8)	4.5	2.2-9.1	0.000
One sexual partner whole life	95 (96.9)	193 (93.2)	0.4	0.2-0.9	0.034

N, number; OR, Odds ratios; CI, Confidence interval

Table 5
Adjusted risk factors for ectopic pregnancy.

Variables	Case (*N*=113) No. (%)	Control (*N*= 226) No. (%)	Crude OR	95% CI	*p*-value	Adjusted OR	95% CI	*p*-value
Prior ectopic								
No	70 (71.4)	205 (99.0)						
Yes	28 (28.6)	2 (1.0)	0.02	0.01-0.1	0.000	28.3	5.8-138	0.0005
Prior spontaneous abortion								
No	89 (90.8)	161 (77.8)						
Yes	9 (9.2)	46 (22.2)	2.83	1.3-6.0	0.006	0.3	0.1-0.7	0.011
Contraception								
No	40 (40.8)	15 (7.2)						
Yes	58 (59.2)	192 (92.8)	8.83	4.6-17.1	0.000	0.097	0.04-0.23	0.0005
History of STI								
No	20 (20.4)	155 (74.9)						
Yes	78 (79.6)	52 (25.1)	0.86	0.05-0.15	0.000	11.06	5.45-22.44	0.0005

OR, Odds ratio; CI, Confidence interval

of the 3 infections and a past history of ectopic pregnancy or past history of STI symptoms.

Among cases, chlamydia tests were positive in 17 (15.0%) tubal PCR specimens, 9 (8.0%) cervical PCR specimens and 6 (5.3%) IgM ELISA assays (Table 6). Five cases were positive for all 3 infections. Five cases were positive for 2 infections and 7 cases were positive for one test (tubal PCR). Chlamydia IgM was positive only in cases that were also positive on tubal or cervical PCR. Similarly, all positive cervical PCR cases were also positive on tubal PCR or IgM serology. Gonorrhea DNA was detected in 4 cervical swabs but only one tubal specimen (results not shown).

Table 6
Chlamydia PCR and IgM results in women with ectopic pregnancy.

Case No.	Tubal chlamydia	Cervical chlamydia	Chlamydia IgM
5	+	+	+
7	+	-	-
17	+	+	-
25	+	-	-
33	+	-	+
36	+	+	-
44	+	-	-
45	+	+	+
46	+	+	+
61	+	+	-
71	+	+	+
73	+	-	-
77	+	-	-
81	+	-	-
94	+	+	-
98	+	-	-
101	+	+	+
Total	17	9	6

+, positive; -, negative
A positive result for chlamydia IgM = reading >OD0.250

DISCUSSION

In this study, the variables positively associated with ectopic pregnancy were previous tubal surgery, lower abdominal surgery, ectopic pregnancy, spontaneous abortion, or infertility. All these factors could have an infectious etiology since tubal damage often results from ascending infection of the lower genital tract. Untreated or inadequately treated infection can progress to tubal damage requiring tubal surgery, or result in complications of reproduction, such as spontaneous abortion, infertility or ectopic pregnancy. The association between a previous history of ectopic pregnancy or STI symptoms and current ectopic pregnancy was particularly strong as has been seen inother studies (Chow *et al*, 1987; Coste *et al*, 1991; Ankum *et al*, 1996; Bouyer *et al*, 2003; Mascrenhas, 2004).

Having multiple partners is usually identified as a risk factor for ectopic pregnancy in studies of STIs. In this study, nearly all participants claimed to have only one lifetime sexual partner. Previous studies from Myanmar (Zarni-Win, 2008) have reported similar findings. The accuracy of these findings cannot be ascertained, since women from a conservative Asian culture are usually reluctant to disclose their sexual behavior. Data obtained regarding induced abortion may also have been biased since induced abortion is illegal in Myanmar, hence, admission of this act would incriminate the subject.

C. trachomatis and *N. gonorrhoeae* are generally considered the most important

bacterial causes of PID leading to tubal pathology and ectopic pregnancy (Ho *et al*, 1992; Mahony *et al*, 1992). The incidence of ectopic pregnancy in Western countries has increased along with an increase in chlamydia infection (Mascarenhas, 2004). In the United Kingdom, comprehensive programs to prevent chlamydia have not only decreased the incidence of chlamydia infection but also the rate of ectopic pregnancy (Egger *et al*, 1998). In developing countries, there is evidence chlamydia is more prevalent than gonorrhea in both lower and upper genital tract infections (Ramachandran and Ngeow, 1990).

In this study, *C. trachomatis* was identified more frequently than *N.gonorrhoeae* from cervical swabs from both cases and controls, and in tubal tissue collected from cases at laparotomy. The difference in cervical infection rate between cases and controls was significant for chlamydia but not for gonorrhea. Chlamydia DNA was detected more frequently in tubal tissue than in cervical swabs but the reverse was true for gonorrhea DNA. These findings are consistent with a higher prevalence of chlamydia lower and upper genital tract infections and the indolent, persistent nature of chlamydia infections. Compared to gonorrhea, chlamydia is more often asymptomatic and more likely to go untreated; causing ascending infection and its related sequelae; and to be present with concurrent cervical and tubal infections. In gonococcal salpingitis, the preceding cervical infection is often no longer active. Hence, the cervical swab is less sensitive for diagnosing of gonococcal upper genital tract infection than it is for chlamydia upper genital tract infection.

PCR was chosen to detect chlamydia since this technique causes fewer problems with specimen storage and transportation. A positive PCR is usually taken to indicate the presence of active or recent infection but there is little information regarding the persistence of bacteria DNA following an infection and the sensitivity of the PCR on tubal tissue. Hence, a chlamydia IgM assay was included as an additional diagnostic test to compare the level of exposure to chlamydia between women with ectopic pregnancy and controls. The results showed a higher IgM positivity among cases (5.3%) than controls (4.0%), although the difference was not significant (p=0.365). There were only 6 IgM positives among the 17 tubal PCR positive cases, indicating that a large proportion of the chlamydia tubal infections were not recent or primary infections. In cases where PCR was positive and IgM negative, it was not possible to distinguish between a false positive PCR and persistence of chlamydia DNA beyond the period of IgM seropositivity. Despite limitations, PCR can be recommended for diagnosis of chlamydial genital tract infections in developing countries where there are facilities for molecular diagnosis with adequate quality assurance. In this study, PCR helped establish the importance of *C. trachomatis* as a cause of ectopic pregnancy in Myanmar women.

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