

## Original Article

# *Neisseria gonorrhoea*, *Chlamydia trachomatis*, and *Treponema pallidum* Infection in Antenatal and Gynecological Patients at Korle-Bu Teaching Hospital, Ghana

Kwasi Akyem Apea-Kubi\*, Shinya Yamaguchi<sup>1</sup>, Bright Sakyi<sup>2</sup>, Toshio Kisimoto<sup>3</sup>,  
David Ofori-Adjei<sup>1</sup> and Toshikatsu Hagiwara<sup>3</sup>

Department of Obstetrics and Gynaecology, University of Ghana Medical School, Korle-Bu Teaching Hospital,

<sup>1</sup>Japan International Cooperation Agency, Infectious Disease Expert and

<sup>2</sup>Bacteriology Unit, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana and

<sup>3</sup>National Institute of Infectious Diseases, Tokyo 162-8640, Japan

(Received April 26, 2004. Accepted July 8, 2004)

**SUMMARY:** Five hundred and seventeen women attending the gynecology and obstetrics clinics of the Korle-Bu Teaching Hospital were examined for sexually transmitted infections (STIs). Vaginal swabs were examined for *Trichomonas vaginalis*, *Candida albicans*, and *Gardnerella vaginalis* infection. Endocervical swabs were examined for *Neisseria gonorrhoea* and *Chlamydia trachomatis* using a recently developed RNA detection kit. Strain typing was performed to identify serovars of *C. trachomatis*. Sera were analyzed for *Treponema pallidum* with a passive-particle agglutination assay kit. The prevalence of infection with *N. gonorrhoea* was 0.6%, *C. trachomatis* 3.0%, and *T. pallidum* 5.6%. Eight samples were PCR-positive for *C. trachomatis*. Five of these were serovar G, and the rest were serovar E. All cases of mixed infections occurred in pregnant women. In conclusion, a high transmissible risk of *T. pallidum* infection was observed among our study population and in particular among our pregnant women. The absence of association between the presenting symptoms, clinical findings, and specific pathogens has implications for the syndromic approach to STI case management. The low prevalence of *C. trachomatis* and *N. gonorrhoea* may be due to self medication and requires further research in primary health institutions in rural areas to compare rates.

## INTRODUCTION

This study was undertaken to determine the prevalence of *Neisseria gonorrhoea*, *Chlamydia trachomatis*, and *Treponema pallidum* in our antenatal and gynecological patients.

Sexually transmitted infections (STIs) constitute a major threat to health worldwide, particularly to women and newborns (1). The prevalence is much higher in developing countries, and STIs are ranked among the top ten most important health problems for which adults seek help in outpatient clinics (2). Most STIs are increasing in prevalence and more people are becoming infected with the severer types such as the human immunodeficiency virus (HIV), hepatitis B virus, and the human papillomavirus.

Aside from the economic consequences to society and the individual, STIs facilitate the acquisition and transmission of HIV (3). Pregnancy wastage, low birth weight, birth defects, neonatal infections, prenatal deaths, and premature labor are STIs-associated complications during pregnancy. Infertility, chronic pelvic pain, and ectopic pregnancy are other important sequelae of STIs. Approximately 67% of infertility in sub-Saharan Africa is due to tubal blockage from STIs and other organisms (4,5).

In most African countries, including Ghana, STIs are not

notifiable, and most prevalence studies are based on patients attending either family planning or antenatal clinics in urban areas, a situation that in part accounts for the widely divergent results from various researchers.

## PATIENTS AND METHODS

The study was carried out between November 2000 and December 2001 on patients attending gynecological and antenatal clinics at the Korle-Bu Teaching Hospital, a 1,600-bed hospital for the University of Ghana Medical School in Accra, the capital city of Ghana. It is the largest hospital in the country.

Two hundred and eighty-four newly registered regular antenatal patients and 233 gynecological patients whose subjective symptoms were abdominal pain, genital bleeding, vaginal discharge, and dysuria and who gave informed consent were enrolled consecutively into the study. Three patients declined participation.

A standardized questionnaire administered by trained STI nurse/counselors asked about demographic and behavioral factors and current symptoms including vaginal discharge. After general examination, a bivalve vaginal speculum was passed. No antiseptic lotions or creams were used for lubrication and where necessary the vaginal speculum was moistened with sterile water. Excess cervical and vaginal secretions were removed with large cotton-wool swabs before two cotton-tipped swabs were introduced into the cervical canal simultaneously (where feasible) and gently rotated, without undue discomfort to the patient, and then withdrawn. The endocervical swabs were placed immediately into sucrose

\*Corresponding author: Mailing address: Department of Obstetrics and Gynaecology, University of Ghana Medical School, Korle-Bu Teaching Hospital, Korle-Bu, Accra, Ghana. Tel: +233-21- 661347, +233-20-8150297, Fax: +233-21-668425, E-mail: K\_apeakubi@hotmail.com

phosphate glutamate (SPG) transport medium, labeled, and transported in coolers, first to the Bacteriology Unit of the Noguchi Memorial Institute for Medical Research (NMIMR), Accra, and then to the National Institute of Infectious Diseases (NIID), Tokyo, Japan, for *C. trachomatis* and *N. gonorrhoea* detection.

Five milliliters of blood was collected by venipuncture of a cubital vein from each of the 517 participants, labeled, and transported in coolers to the Virology Unit of the NMIMR for *T. pallidum* detection.

#### Pathogen identification:

*N. gonorrhoea* and *C. trachomatis*: A total of 465 original SPG samples were transported on dry ice to the NIID, for detection of *C. trachomatis* and *N. gonorrhoea* ribosomal RNA (rRNA) using the APTIMA Combo 2 Assay (Gen-Probe Inc., San Diego, Calif., USA), which combines the technologies of target capture, Transcription-Mediated Amplification (TMA), and Dual Kinetic Assay (DKA). Qualitative testing protocols were applied according to the manufacturer's instructions (6). The method of strain-typing by PCR and restriction fragment length polymorphism was performed according to the method of Yoshida and colleagues to identify serovars of *C. trachomatis* (7).

*Treponema pallidum*: The venous blood was separated on the same day and serum tested with SERODIA passive agglutination assay kits (FUJIREBIO Inc., Tokyo, Japan) for *T. pallidum* hemagglutination (TPHA) antibody. The cutoff serum dilution was 1:80.

The study was approved by the Ethical Review of Research Committee of the University of Ghana, Accra, Ghana.

**Statistical analysis:** The frequency data were analyzed by chi-square or Fisher's exact tests using EPI-Info 2002 software (Centers for Disease, Control and Prevention, Atlanta, Ga., USA). A *P* value < 0.05 was considered statistically significant.

## RESULTS

A total of 517 women were recruited into the study. The age groups and marital status of the participants are shown in Table 1. The age and marital status of 17 patients were not recorded. The mean age was 29.6 years (SD = 6.9). Four hundred and one (77.6%) patients were aged 35 and below, and 28 (4.2%) above 41 years. A total of 315 (62.9%) patients received secondary school education and 60 (12%) had no formal education. Fifteen patients did not provide information on parity; 300 (59.8%) patients had one or more children.

Table 2 shows the presenting symptoms among gynecological patients. There was no statistically significant association between presenting symptoms and either chlamydia or gonococcal infection. In terms of association between presenting symptoms and clinical observation, 184 (82.5%) women who complained of vaginal discharge actually had the discharge on clinical examination, and 161 (59.0%) who did not report vaginal discharge showed abnormal discharge ( $\chi^2 = 27.4$ ; *P* < 0.01).

The prevalence of infective agents by pregnancy status is shown in Table 3. Regarding the association among the four presenting symptoms, lower abdominal pain was significantly associated with vaginal discharge ( $\chi^2 = 17.6$ ; *P* < 0.01) and with dysuria ( $\chi^2 = 11.4$ ; *P* < 0.01).

Of the eight samples that were PCR-positive for *C. trachomatis*, five were serovar G, and the rest were serovar E.

Table 1. Age group and marital status of study subjects

Age (yrs)	Married	Divorced/ widowed	Not married	Total
16-20	24		9	33
21-25	104		20	124
26-30	133	5	6	144
31-35	90	5	5	100
36-40	62	5	4	71
41+	21	7		28
Total	434	22	44	500

Table 2. Presenting symptoms among gynecological patients

	Gynecological patients <i>n</i> = 223 (%)
Vaginal bleeding	25 (11.2)
Low abdominal pain	131 (58.7)
Vaginal discharge	111 (49.8)
Dysuria <sup>1)</sup>	20 (9.4)
At least one symptom	182 (81.6)

<sup>1)</sup>: Ten gynecological patients were excluded because of missing information.

Table 3. The prevalence of pathogens by pregnancy status

	Pregnant (%)	Non-pregnant (%)	Total prevalence (%)
<i>T. pallidum</i>	21/294 (7.1)	8/223 (3.6)	29/517 (5.6)
<i>C. trachomatis</i>	9/261 (3.4)	5/204 (2.5)	14/465 (3.0)
<i>N. gonorrhoea</i>	1/261 (0.4)	2/204 (1.0)	3/465 (0.6)

All four cases of mixed infections involved pregnant women, as follows: two cases of *C. trachomatis* and *Trichomonas vaginalis*, two of *C. trachomatis* and *Candida albicans*. One patient with *C. trachomatis* infection tested positive for HIV.

## DISCUSSION

Syphilis remains a significant cause of preventable perinatal death in many developing countries. Worldwide, many pregnant women are not tested for syphilis infection, and available data indicate that the incidence of adverse pregnancy outcome attributable to syphilis in Africa is the highest in the world (8). Between 3-18% of pregnant women in sub-Saharan Africa are infected with syphilis (9), which results in fetal or infant death or morbidity for 50-80% of affected pregnancies (10). Serological tests for syphilis using rapid plasma reagin (RPR) and TPHA in the Northern, Upper East, and Upper West regions of Ghana revealed a prevalence rate for syphilis of 8-18% (11). A potential problem with most of the reported studies from Africa is that non-specific serologic tests like RPR and the venereal disease research laboratory (VDRL) that show sero-reactivity to yaws and endemic syphilis were used to assess the prevalence rates in these countries. Further, the interpretation of these serological tests is made even more difficult by reports from sub-Saharan Africa where specific treponemal tests yielded much higher prevalences than non-treponemal tests in the same women. This observation was confirmed in Zambia where the RPR false-negative rate ranged between 11 and 14% (12).

In this study, 29 out of 517 patients (5.6%) tested positive

on the specific TPHA test, which contrasts with the 13.6% seroprevalence among blood donors in Ghana. In Indola, Zambia, the prevalence of syphilis sero-reactivity was over 10% in both men and women; 6% in Yaounde, Cameroon; 3-4% in Kisumu, Kenya; and 1-2% in Cotonou, Benin (13). These values are remarkably higher than that in a report from the United Kingdom, which showed a very low 0.03% (15 in 58,445) prevalence of syphilis in pregnant women over a 6-year period (14). We found our *T. pallidum* prevalence rate to be comparable to that in Cameroon and Kenya, and demonstrated a high transmissible risk of *T. pallidum* among our study population and in particular in our pregnant women. A more effective national antenatal screening and intervention strategies are needed to prevent maternal and fetal morbidity and mortality.

Gonorrhoea is usually transmitted by sexual intercourse. The reported risk to a male of acquiring the infection after a single exposure to an infected female is 22-50%. The risk to a female after similar exposure to an infected male is 60-90%, and increases to 100% with more than two exposures (15). There is overwhelming evidence that gonococcal infection facilitates HIV transmission.

Conventional diagnosis of *N. gonorrhoea* infection requires isolation of the organism on selective media or the observation of diplococci in Gram-stained smears. Culture results can have good clinical sensitivity but are highly dependent on proper specimen collection and handling. Improper specimen storage and transport can result in the loss of organism viability and a false-negative culture result. In addition, poor sampling technique, toxic sampling materials, and the inhibition of growth by components of body secretions can also result in a false-negative result (5). Commonly used non-culture methods for *N. gonorrhoea* detection include direct DNA assays. However, technological issues such as cumbersome specimen processing and specimen inhibition that can yield false-negative results have limited the use of first generation Nucleic Acid Amplification (NAA) test for *C. trachomatis* and *N. gonorrhoea* (5). The APTIMA Combo 2 Assay is a second generation NAA test that utilizes target capture, TMA, and DKA to streamline specimen processing, amplify target rRNA, and detect amplicons, respectively. The APTIMA Combo 2 Assay qualitatively detects *C. trachomatis* and/or *N. gonorrhoea* rRNA in endocervical and male urethral swab specimens, and in male and female specimens from symptomatic and asymptomatic individuals.

The reported prevalences of gonorrhoea in African antenatal clinics have been 0% in Nairobi, 6.7% in Bakau, and 14% in Yaoundé (3). In 1985, Bentsi and colleagues reported that 5 out of 162 (3.1%) gynecological patients in the same hospital were infected with *N. gonorrhoea* (16). The pathogen was isolated from 32.2% (106/329) of commercial sex workers in Accra (personal communication, Program Manager, Ghana AIDS Control Program). The 0.6% prevalence of gonorrhoea in our study compares with results in recently published studies from four cities in sub-Saharan Africa that ranged between 0% in Kisumu and 2.7% in Yaoundé (13). It is equally important to note the decline in gonorrhoea in Yaoundé from 14 to 2.7% during the 21-year period from 1980 to 2001. The low gonococcal rRNA detection rate (0.6%) suggests that the prevalence of *N. gonorrhoea* in antenatal and gynecological patients in Accra is low. We are unable to explain why except that most of our patients had received treatment (the precise nature was unknown) before referral. In this survey, 69% of gynecologi-

cal patients and 30% of pregnant women (data not shown) had received some treatment since the onset of subjective symptoms. The observed prevalence rate may not be applicable in the rural areas of Ghana, where people may not have easy access to anti-biotics. There is, therefore, a need for further research in these areas to test the above hypothesis. Self-medication with antimicrobials is common in Ghana and probably the rest of Africa. The findings of Arya and colleagues, which showed that 40% of pregnant women in the Teso district of Uganda were infected with *N. gonorrhoea* and that among those of Widy-Wirski and D'Costa 27.5% of all women in one rural village in the Central African Republic had gonorrhoea (4), suggest that the prevalence may indeed be higher in rural areas. Although we analyzed the association between the patient's prior treatment and *N. gonorrhoea*, we could not establish a significant association. This may in part be due to the small number of cases of *N. gonorrhoea* and the small sample size.

Several methods for *C. trachomatis* detection have been utilized in clinical laboratories, including direct fluorescent antibody testing, enzyme immunoassay, and cell culture, the last of which was once considered the 'gold standard' for detection of *C. trachomatis*. Although culture is quite specific, recent reports have demonstrated the sensitivity and specificity of nucleic acid amplification tests to be superior to that of culture and direct specimen tests (5).

The first reported attempt to isolate *C. trachomatis* from the cervixes of patients in Ghana was in 1985, at which time 8 (4.9%) of 162 gynecological patients and 3 (7.7%) of 39 postpartum women were found to be infected with the pathogen (16). In 1998, *C. trachomatis* was isolated from 10% (33/329) of commercial sex workers in Accra (personal communication, Program Manager, Ghana AIDS Control Program). The pathogen was isolated from 6.7% of antenatal patients in Bakau, 6% of antenatal patients in Nairobi, and 13 and 16% of women attending an STI clinic and family planning clinic, respectively, in Johannesburg (4).

In a *C. trachomatis* infection prevention program, the prevalence was shown to have declined from 10-12% in the late 1980s to only 3-5% by 1995 (17).

The 3% prevalence of *C. trachomatis* in Accra is comparable to that found in women in Cotonou, Benin (1.3%), and in Kisumu, Kenya (4.5%) (13). *C. trachomatis* serotypes G (5/8) and E (3/8) contrast with an earlier study in Accra in which all three isolates of *C. trachomatis* were serovar G (16). Takahashi and colleagues reported *C. trachomatis* serovar D (29%), E (21%), and G (19%) as the most common among genital serovars in Japan (18).

This study has described the current status of STI pathogens, especially *N. gonorrhoea*, *C. trachomatis*, and *T. pallidum* in gynecological and obstetrics patients in a tertiary hospital in Ghana. The low prevalence of *C. trachomatis* and *N. gonorrhoea* may have been the result of self-medication, and calls for further research in primary health institutions in rural communities to compare the rates. The rather small sample size and low pathogen prevalence limited the ability to detect significant differences. However, the absence of association between the presenting symptoms, clinical findings, and specific pathogens has implications for the syndromic approach to STI case management. The high prevalence of *T. pallidum* infection in our pregnant women calls for a more effective national antenatal screening and intervention strategies to prevent fetal morbidity and mortality from congenital syphilis.

## ACKNOWLEDGMENTS

We are grateful to the Japan International Cooperation Agency (JICA) Infectious Disease Project at Noguchi Memorial Institute for Medical Research for financial and technical support. We also wish to acknowledge Drs. Yan Cai, Motohiko Ogawa, and Tsutomu Yamazaki for their assistance with *C. trachomatis* and *N. gonorrhoea* isolation.

## REFERENCES

1. Cates, W., Farley, T. M. M. and Rowe, P. J. (1985): World-wide patterns of infertility: is Africa different? *Lancet*, ii, 596-598.
2. Over, M. and Piot, P. (1990): HIV infection and other sexually transmitted diseases. In Jamison, D. T. and Mosley, W. H. (eds.), *Evolving Health Sector Priorities in Developing Countries*. vol. 1. p. 87. World Bank, Washington, D.C.
3. Laga, M. (1995): STD control for HIV prevention - it works! *Lancet*, 346, 518.
4. Mabey, D. C., Lloyd-Evans, N. E., Conteh, S. and Forsey, T. (1984): Sexually transmitted diseases among randomly selected attenders at an antenatal clinic in The Gambia. *Br. J. Vener. Dis.*, 60, 331-336.
5. De Muylder, X., Laga, M., Tennstedt, C., Van Dyck, E., Aelbers, G. N. M. and Piot, P. (1990): The role of *Neisseria gonorrhoea* and *Chlamydia trachomatis* in pelvic inflammatory disease and its sequelae in Zimbabwe. *J. Infect. Dis.*, 162, 501-505.
6. Gen-Probe Incorporated (2002): Gen-Probe APTIMA Combo 2 Assay. For in vitro diagnostic use. IN003 Rev. B/2002-06 c 2001. Gen-Probe Incorporated, San Diego.
7. Yoshida, H., Kishi, Y., Shiga, S., Inouye, S. and Hagiwara, T. (1995): Serotyping of *Chlamydia trachomatis* by polymerase reaction. *Jpn. Arch. Sex. Trans. Dis.*, 6, 40-45.
8. Myer, L., Wilkinson, D., Lombard, C., Zuma, K., Rotchford, K. and Abdool Karim, S. S. (2003): Impact of on-site testing for maternal syphilis on treatment delays, treatment rates, and perinatal mortality in rural South Africa: a randomised controlled trial. *Sex. Transm. Infect.*, 79, 208-213.
9. Genc, M. and Ledger, W. J. (2000): Syphilis in pregnancy. *Sex. Transm. Infect.*, 76, 73-79.
10. Gloyd, S., Chai, S. and Mercer, M. (2001): Antenatal syphilis in sub-Saharan Africa: Missed opportunities for mortality reduction. *Health Policy Plan.*, 16, 29-34.
11. Disease Control Unit, Ministry of Health (1994): HIV/STD Sentinel Surveillance. p. 7. Ministry of Health, Accra, Ghana.
12. Schulz, K. F., Murphy, F. K., Patamasucon, P. and Maheus, A. Z. (1990): Congenital syphilis. p. 824-825. In Holmes, K. K., Mårdh, P-A., Sparling, P. F., Wiesne, P. J., Cates, W. Jr., Lemon, S. M. and Stamm, W. E. (eds.), *Sexually Transmitted Diseases*. 2nd ed. McGraw-Hill Information Services Co., New York.
13. Buve, A., Weiss, H. A., Laga, M., Van Dyck, E., Musonda, R., Zekeng, L., Kahindo, M., Anagonou, S., Morrison, L., Robinson, N. J., Hayes, R. J. and Study Group on Heterogeneity of HIV Epidemics in African Cities (2001): The epidemiology of gonorrhoea, chlamydial infection and syphilis in four African cities. *AIDS*, 15 Suppl. 4, S79-88.
14. Cameron, S. T., Thong, K. J., Young, H. and Liston, W. A. (1997): Routine antenatal screening for syphilis in Lothian: a study of the results 1988 to 1994. *Br. J. Obstet. Gynaecol.*, 104, 734-737.
15. Platt, R., Rice, P. A. and McCormack, W. M. (1983): Risk of acquiring gonorrhoea and prevalence of abnormal adnexal findings among women recently exposed to gonorrhoea. *JAMA*, 250, 3205-3209.
16. Bentsi, C., Klufio, C. A., Perine, P. L., Bell, T. A., Cles, L. D., Koester, C. M. and Wang, S. P. (1985): Genital infections with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Ghanaian women. *Genitourin. Med.*, 61, 48-50.
17. Marrazzo, J. M., Celum, C. L., Hillis, S. D., Fine, D., DeLisle, S. and Handsfield, H. H. (1997): Performance and cost-effectiveness of selective screening criteria for *Chlamydia trachomatis* infection in women. Implications for a national Chlamydia control strategy. *Sex. Transm. Dis.*, 24, 131-141.
18. Takahashi, K., Yoshida, H., Hagiwara, T. and Sato, K. (1998): Serovar distribution of genital *Chlamydia trachomatis* in Japanese women, and its correlation with clinical symptoms. *J. Infect. Chemother.*, 4, 32-35.