The Role of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in Pelvic Inflammatory Disease and Its Sequelae in Zimbabwe

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The presence of antibodies to pili of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* serovar L2 were assessed in women consecutively hospitalized in Zimbabwe with pelvic inflammatory disease (PID; $n = 66$), infertility ($n = 227$), and ectopic pregnancy ($n = 60$). Women delivering live full-term infants served as controls. Of the infertile women, 60% had secondary infertility; 59% had macroscopic evidence of a tubal abnormality. Women with PID, infertility and tubal disease, and ectopic pregnancy and tubal disease had significantly higher prevalences of antibodies against *C. trachomatis* and *N. gonorrhoeae* than did controls or women with infertility or ectopic pregnancy but no macroscopic tubal abnormalities ($P < .001$ for all comparisons). The prevalence of antibody to chlamydia increased with age ($P = .01$), unlike the gonococcal antibody. Antibodies to *C. trachomatis* were associated with a history of PID and with antibody to *N. gonorrhoeae*. Gonococcal antibody correlated with a history of PID, being single, a positive *Treponema pallidum* hemagglutination assay, and chlamydial antibody. None of the controls had human immunodeficiency virus, unlike 3.9%–7.6% of the other women. Tubal abnormalities were implicated in more than half of the cases of infertility.

Pelvic inflammatory disease (PID) and infertility are leading causes of admission to gynecologic wards in many areas of Africa [1, 2] where diagnosis and treatment of these conditions remain a major public health challenge.

Infertility and ectopic pregnancy are both recognized consequences of tubal dysfunction due to previous pelvic inflammatory disease [3]. In some areas, >20% of African women are involuntarily childless [4]. Moreover, the proportion of infertility cases that is attributable to past infections is estimated at ~85% in Africa in contrast to 20%–40% elsewhere [5]. Although no population-based incidence data on ectopic pregnancies in developing countries are available, ectopic pregnancy is thought to be common and remains associated with a high mortality rate, especially in rural areas where easily accessible critical care facilities are often lacking.

Chlamydia trachomatis is the most important cause of PID in the industrialized world [6], but the relative roles of *C. trachomatis* and *Neisseria gonorrhoeae* in causing PID in developing countries are unknown [7].

Many surveys in Africa have found a high prevalence of gonococcal infections among women receiving antenatal care (1%–15%) [8–11]. Some recent reports suggest that genital chlamydial infections are also highly prevalent and may outnumber gonococcal infections [8–11].

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Our study was undertaken to assess the relative importance of *C. trachomatis* and *N. gonorrhoeae* in the etiology of PID, tubal infertility, and ectopic pregnancy in a rural area in Zimbabwe. Exposure to syphilis and human immunodeficiency virus (HIV) in different groups of women was also studied.

Methods

Study population. We studied women consecutively admitted to Gweru Provincial Hospital, Zimbabwe, from 1985 to 1987. Of these, 66 had PID and peritonitis, 227 had a history of infertility, and 60 had ectopic pregnancies. Also, 104 women who delivered normal full-term infants were recruited randomly during the study period and were considered the control population. Nineteen women who delivered stillborn infants were also included.

PID was diagnosed when three signs were present: acute onset of lower abdominal pain with vaginal discharge or dysuria, fever, and rebound tenderness or cervical motion.

Sixty laparotomy-proven cases of ectopic pregnancy were divided into two subgroups according to pelvic status. Thirty-nine patients had pelvic findings suggestive of previous PID, including gross adhesions, contralateral hydrosalpinx, and previous surgery for tubal infertility. Twenty-one patients had macroscopically normal pelvic findings on laparoscopy. Tubal tissues were not examined histologically.

Two hundred twenty-seven patients, infertile >18 months, were divided into two groups by tubal conditions: 135 had obvious tubal pathology as demonstrated by hysterosalpingography (98), laparoscopy (28), or laparotomy (9); 92 women had normal fallopian tubes, (80 by hysterosalpyngography, 5 by laparoscopy, and 7 by both examinations).

Characteristics such as age, marital status, parity, and history of PID were collected from all patients. Mild PID was defined as an

Table 1. Characteristics of the women studied.

Group	No.	Mean age (± SD)	No. married (%)	No. nullipara (%)	History of PID: Mild (%)	History of PID: Severe (%)
At term intrauterine pregnancy	104	26.6 (± 5.6)	91 (87.5)	27 (26)	12 (12)	1 (1)
PID	66	26.9 (± 6.2)	47 (71.2)	13 (20)	42* (65)	8† (12)
Ectopic pregnancy						
Tubal pathology	39	28.9‡ (± 5.9)	33 (89.7)	6 (15.4)	24* (65)	8* (22)
Normal tubes	21	27.6 (± 5.3)	21 (100)	1 (4.8)	1 (6)	0
Infertile with:						
Tubal pathology	135	28.4‡ (± 4.8)	135 (100)	54§ (40)	105* (81)	4 (3)
Normal tubes	92	27.1 (± 4.9)	92 (100)	40§ (43)	28† (30)	0
Stillborn birth	19	24.8 (± 6.4)	12‖ (63.2)	4 (21)	8* (44)	1 (6)

NOTE. PID = pelvic inflammatory disease.
* $P < .001$ (χ^2 test) compared with controls.
† $P < .01$ (χ^2 test) compared with controls.
‡ $P < .05$ (Student's t test) compared with controls.
§ $P < .05$ (χ^2 test) compared with controls.
‖ $P = .06$ (χ^2 test) compared with controls.

episode of PID without hospitalization; severe PID was defined as an episode of PID that required hospitalization because of signs of peritonitis.

Blood was collected from each patient (10 ml), and serum samples were stored at −20°C until serologic testing was performed in Europe.

Serology. Chlamydial serology was performed by single antigen indirect immunofluorescence test using *C. trachomatis* L2 serovar–infected McCoy cells [12]. Gonococcal serology was performed by enzyme-linked immunosorbent assay (ELISA). Purified gonococcal pili from strain 6650 were used as antigen [13].

Serologic tests for syphilis included the rapid plasma reagin test (RPR; Becton, Dickinson, Baltimore) and the *Treponema pallidum* hemagglutination assay (TPHA; Fujirebio, Tokyo). HIV IgG antibody was determined in an ELISA (Organon Teknika, Oss, Netherlands), followed by a Western blot assay (DuPont de Nemours, Brussels) on sera reactive in the ELISA. Specimens containing at least one band representative of the HIV core proteins and one band representative of the envelope glycoproteins were considered positive for HIV antibody.

Statistical analysis. Statistical analysis was performed using the SPSS statistical package. Univariate analysis was done for categorical variables using the χ^2 test and one-tailed Fisher's exact test; for continuous variables Student's t test was used. The odds ratio (as an estimate of relative risk in a retrospective study) was calculated as a cross-product ratio: [(exposed − diseased) × (unexposed − not diseased)]/[(unexposed − diseased) × (exposed − not diseased)]. Cornfield 95% confidence limits of the odds ratios were used.

Results

Demographic characteristics. Table 1 summarizes the characteristics of the six different study groups. In all groups the mean ages were similar to the control population (26.6 years) except for women with ectopic pregnancy and infertile women (both with tubal disease), whose mean ages were slightly higher (28.9 and 28.4 years, respectively; $P < .05$). Women with PID and those who delivered a stillborn infant were more likely to be single than were pregnant controls. The frequency of nulliparity was similar in the different groups, except for infertile women. Of infertile women with and without tubal disease, 40% and 43%, respectively, were nulliparous, indicating that 60% had secondary infertility.

Control women had a history of PID less often than those in other groups. A history of PID was reported more frequently by women with current PID, by women with ectopic pregnancy and tubal disease, and by infertile women with tubal disease (77%–87%).

Prevalence of antibodies to C. trachomatis *and* N. gonorrhoeae. Table 2 shows the percentage of women with chlamydial and gonococcal antibodies among controls, women with PID, and infertile women with and without tubal disease.

Significantly higher rates of chlamydial antibodies were found in women with PID (68.2%, titer ≥1:16 and 25.8%, titer ≥1:64) and infertile women with tubal disease (77%, titer ≥1:16 and 38.5%, titer ≥1:64) as compared to controls (42%, titer ≥1:16 and 6.7%, titer ≥1:64; $P < .001$). Infertile women without tubal disease had rates similar to controls.

About half of the women with PID (54.5%) and of the infertile women with tubal abnormalities (51.1%) had antibody to *N. gonorrhoeae* versus 4.8% of the controls ($P < .001$). Infertile women with normal tubes also had a fivefold higher prevalence of gonococcal antibodies (25%) than controls ($P < .01$), although this rate was lower than among women with PID or infertile women with abnormal tubes. In all instances, odds ratios for gonococcal antibodies were consistently higher than for chlamydial antibodies. Among women with gonococcal antibodies and infertility, 71% had a history of PID; among those with gonococcal antibodies and ectopic pregnancy 63% had a history of PID.

Chlamydial (titer ≥1:64) and gonococcal antibodies were concomitantly present in 1.9% of controls versus 13.6% of

Table 2. Prevalence of antibodies to *Chlamydia trachomatis* and *Neisseria gonorrhoeae* among women presenting with pelvic inflammatory disease (PID) or infertility versus a control group with intrauterine pregnancy.

Group	Chlamydia titer		Gonococcus	Chlamydia ≥1:64 + gonococcus
	≥1:16	≥1:64		
Controls, n = 104	44 (42)	7 (6.7)	5 (4.8)	2 (1.9)
PID, n = 66	45 (68.2)*	17 (25.8)*	36 (54.5)*	9 (13.6)*
OR, 95% CI	2.9, 1.5–5.9	4.8, 1.7–13.8	23.8, 8.0–76.2	8.1, 1.5–56.0
Infertile women with				
Normal tubes, n = 92	33 (36)	5 (5.4)	23 (25.0)†	1 (1.1)
Abnormal tubes, n = 135	104 (77)*	52 (38.5)*	69 (51.5)*	26 (19.3)*
OR, 95% CI	4.6, 2.5–8.3	8.7, 3.6–22.2	20.7, 7.5–61.7	12.2, 2.7–76.1

NOTE. OR = odds ratio, CI = confidence interval. Data are number (%) of women.
* $P < .001$ compared with controls.
† $P < .05$ compared with controls; OR, 8.3, 95% CI, 2.6–29.6.

Table 3. Prevalence of antibodies to *Chlamydia trachomatis* and *Neisseria gonorrhoeae* among women with ectopic pregnancy with and without tubal disease versus women with intrauterine pregnancy.

Antibodies to, titer	No. (%) with intrauterine pregnancies (n = 104)	No. (%) with ectopic pregnancy		
		Normal tubes* (n = 21)	Abnormal tubes (n = 39)	OR, 95% CI
C. trachomatis				
≥1:16	44 (42)	7 (33)	26 (66.7)†	2.7, 1.2–6.4
≥1:64	7 (6.7)	1 (4.8)	10 (25.6)‡	4.8, 1.5–15.5
N. gonorrhoeae	5 (4.8)	3 (14.3)	13 (33.3)‡	12.4, 3.4–49.6
Chlamydia (≥1:64) + gonococci	2 (1.9)	0	5 (12.8)†	7.5, 1.2–58.9

NOTE. OR = odds ratio, CI = confidence interval.
* Macroscopically normal tubes.
† $P < .05$ compared with controls.
‡ $P < .001$ compared with controls.

those with PID and 19.3% of infertile women with abnormal tubes.

Women with ectopic pregnancy and abnormal tubes had chlamydial antibodies more often (66.7%, titer ≥1:16 and 25.6%, titer ≥1:64) than controls and women with ectopic pregnancy but no tubal abnormalities (table 3). Likewise, the prevalence of gonococcal antibodies among women with ectopic pregnancy with tubal disease (33.3%) was six times higher than the control group (odds ratio 12.4). Chlamydial (≥1:64) and gonococcal antibodies were present in 12.8% of women with ectopic pregnancy and abnormal tubes versus 1.9% in controls ($P < .001$).

Association of chlamydial and gonococcal antibodies with other variables. When all study groups (427 patients) were analyzed together, the presence of chlamydial antibodies correlated with older age for titer ≥1:16 (<20 years, 38%; ≥20 to <30 years, 57%; ≥30 years, 64%; $P = .01$) but not for a titer ≥1:64 (<20 years, 15%; ≥20 to <30 years, 21%; ≥30 years, 20%; $P = .6$). There was no relationship between age and the presence of gonococcal antibodies.

Chlamydial antibodies (≥1:64) were more frequently found among women who reported a history of PID (32% vs. 7%, $P < .001$) and among women who had antibodies against *N. gonorrhoeae* ($P = .009$). There was no association with marital status and positive RPR or TPHA results. Gonococcal antibodies correlated strongly with a history of PID (46% vs. 20%; $P = .003$), being single ($P = .03$), a positive TPHA ($P = .001$), and the presence of chlamydial antibodies ($P = .009$). There was no association between gonococcal antibodies and active syphilis (positive RPR and TPHA). These associations remained when pregnant control women were analyzed separately.

Among the infertile women, the prevalence of gonococcal and chlamydial antibodies was similar in the primary versus secondary infertile women.

In a stratified analysis controlling for age, a positive TPHA, and the presence of either gonococcal or chlamydial antibodies, the associations between the different conditions and gonococcal and chlamydial antibodies remained independently significant.

Prevalence of syphilis and HIV. Twelve (63%) of 19 women with stillborn infants had evidence of active syphilis (RPR and TPHA positive) as compared with 8.7% of pregnant control women (who delivered a full-term live infant)

Table 4. Prevalence of syphilis and human immunodeficiency virus (HIV) among women studied in Zimbabwe.

Group	No. TPHA positive (%)	OR, 95% CI	No. RPR and TPHA positive (%)	OR, 95% CI	No. HIV positive (%)
Controls, n = 104	20 (19)		9 (8.7)		0
PID, n = 66	27 (40.9)*	2.9, 1.2–4.1	15 (22.7)*	3.1, 1.2–8.3	5 (7.6)†
Infertility, n = 227	79 (34.8)*	2.2, 1.2–4.1	30 (13.2)*		9 (3.9)†
Ectopic pregnancy, n = 60	24 (40)*	2.8, 1.3–6.1	12 (20)*		4 (6.6)†
Women with stillbirths, n = 19	14 (74)‡	11.8, 3.4–43	12 (63)‡	18.1, 5.0–68.8	1 (5.3)

NOTE. OR = odds ratio, CI = confidence interval, TPHA = *Treponema pallidum* hemagglutination assay, RPR = rapid plasma reagin test.
* $P < .01$ compared with controls.
† $P < .05$ compared with controls (Fisher's exact text).
‡ $P < .001$ compared with controls.

($P < .001$). Women with PID, infertility, and ectopic pregnancy also had higher prevalences of RPR- and TPHA-positive than controls ($P < .01$, table 4).

None of the women who delivered live infants was positive for HIV antibody, versus 7.6% of the women with PID, 3.9% of infertile women, and 6.6% of women with ectopic pregnancy ($P < .05$).

Discussion

This study confirms previous findings that tubal abnormalities are implicated in well over half of all cases of infertility in women in Africa [5]. We also found that women with PID and women with infertility or ectopic pregnancy associated with tubal disease had a much higher prevalence of IgG antibody against *C. trachomatis* and *N. gonorrhoeae* than did control pregnant women. This suggests that these women suffered more frequent, more prolonged, or more severe infections than did pregnant controls and that both *C. trachomatis* and *N. gonorrhoeae* are important causes of tubal sequelae following salpingitis in Zimbabwe.

Several studies from Europe and North America documented an association between infertility with tubal pathology and the presence of circulating antibody to *C. trachomatis* [14–19]. Few investigations, however, have addressed the issue of both gonococcal and chlamydial infection. A study in Bristol, UK, [20] suggested that chlamydiae rather than gonococci are the most identified cause of tubal infertility in the UK, since only 2% of infertile women with tubal pathology had antibodies to gonococcal pili whereas 73% had antibodies to *C. trachomatis*. The low prevalence of gonococcal antibody in the Bristol study may reflect the lower prevalence of gonococcal infections in Western Europe than in Africa because the laboratory methodology was similar in our study. In contrast, Mabey et al., [21] found that both *C. trachomatis* and *N. gonorrhoeae* were equally important causes of infertility due to tubal pathology in a study of 37 women in The Gambia. Our study, based on a larger sample, confirms the hypothesis that both organisms are important causes of PID and its sequelae in Africa.

In Africa, PID and its sequelae have long been thought to be predominantly due to gonococcal infection, and therapy has been mainly directed to this agent. Etiologic studies of PID based on endocervical cultures also have shown higher isolation rates of *N. gonorrhoeae*, varying from 23% in Gabon to 55% in Kenya, than of *C. trachomatis* (15%–21%) [7, 22]. However, since gonococcal PID tends to have a more acute and severe clinical course, whereas an unknown but high percentage of chlamydial PID escapes medical attention, the proportion of gonococcal disease is probably overestimated in hospital-based studies. This may also be the case in our study. Facilities for diagnosing *C. trachomatis* are rare in Africa, and only recently has it been shown that the prevalence of chlamydial infection is at least as high as gonococcal infections in different populations [7–11].

Both chlamydial and gonococcal salpingitis produce high titers of persistent circulating IgG antibody but since the sensitivity of two assays for prior infection may differ because of different methodologies, one can conclude from our serologic results only that both organisms are important causes of PID but not which organism most frequently caused PID.

Well over half of the women with ectopic pregnancy and infertility had tubal scarring suggestive of previous PID. Because the women were consecutively recruited, these proportions may represent a close estimate of the true proportions of cases of ectopic pregnancy and infertility caused by previous PID in Zimbabwe.

Active syphilis was found in nearly 10% of randomly selected pregnant women, which is similar to other studies from southern Africa [8, 23]. Of 19 consecutive women with stillborn babies, 12 had syphilis. This illustrates that syphilis still plays a major role in causing stillbirth in rural Zimbabwe. Syphilis control programs for pregnant women should be initiated immediately in areas without them.

It is clear from this and other studies that both gonococcal and chlamydial infections cause considerable morbidity, in-

cluding PID, infertility, and ectopic pregnancy, in women in developing countries. However, it is not yet clear which control strategies are feasible or affordable in these countries. The growing importance of incurable sexually transmitted viral diseases, such as AIDS, has recently increased the interest in behavior modification and condom use as methods of control, but their impact on the incidence of gonococcal and chlamydial infections remains to be determined. Lack of laboratory-based diagnostic facilities in Africa is a major obstacle to implementing case finding or selective screening programs for diagnosis. Thus, research on simple, rapid, and inexpensive diagnostic tests and operational research on the feasibility of implementing sexually transmitted disease control programs for women are urgently needed.

References

1. Ratnam AV, Din SN, Chatterjee TK. Gonococcal infection in women with pelvic inflammatory disease in Lusaka, Zambia. Am J Obstet Gynecol **1980**;138:965–968
2. Perine PL, Duncan ME, Krause DW, Awoke S. Pelvic inflammatory disease and puerperal sepsis in Ethiopia. I. Etiology. Am J Obstet Gynecol **1980**;138:969–973
3. Weström L. Effect of acute pelvic inflammatory disease on fertility. Am J Obstet Gynecol **1975**;121:707–713
4. Belsey MA. The epidemiology of infertility: a review with particular reference to sub-Saharan Africa. Bull WHO **1976**;54:319–341
5. Cates W, Farley TMM, Rowe PJ. Worldwide patterns of infertility: is Africa different? Lancet **1985**;2:596–598
6. Mårdh PA. Ascending chlamydial infection in the female genital tract. In: Oriel D, Ridgway G, Schachter J, Taylor-Robinson D, Ward M, eds. Chlamydial infections. Cambridge, UK: Cambridge University Press, **1986**:173–184
7. Meheus A, Reniers J, Collet M, Frost E, Leclerc A, Gass R, Ivanoff B. Chlamydia trachomatis in women with acute salpingitis and infertility in Central Africa. In: Oriel D, Ridgway G, Schachter J, Taylor-Robinson D, Ward M, eds. Chlamydial infections. Cambridge, UK: Cambridge University Press, **1986**:241–244
8. Piot P, Meheus A. Lutte contre les maladies sexuellement transmissibles dans les pays en développement. Ann Soc Belge Med Trop **1983**;63:281–311
9. Laga M, Plummer FA, Nsanze H, Namaara W, Brunham RC, Ndinya-Achola JO, Maitha G, Ronald AR, D'Costa J, Bhullar V, Mati JY, Cheang M, Piot P. Epidemiology of ophthalmia neonatorum in Kenya. Lancet **1986**;2:1145–1148
10. Bentsi C, Klufio CA, Perine PL, Bell TA, Cles LD, Koester CM, Wang SP. Genital infections with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Ghanian women. Genitourin Med **1985**;61:48–50
11. Mabey DCW, Lloyd-Evans NE, Conteh S, Forsey T. Sexually transmitted diseases among randomly selected attenders at an antenatal clinic in The Gambia. Br J Vener Dis **1984**;60:331–336
12. Richmond SJ, Caul EO. Fluorescent antibody studies in chlamydial infections. J Clin Microbiol **1975**;1:345–352
13. Oranje AP. Serological diagnosis of gonorrhoea using gonococcal pili as antigen. Thesis, Rotterdam, The Netherlands: Erasmus University, **1986**
14. Padjen A, Nash LD, Viscelli T. The relationship between chlamydia antibodies and involuntary infertility. Fertil Steril **1984**;41(suppl):97S–98S
15. Conway D, Glazener CMA, Caul EO, Hodgson J, Hull MGR, Clarke SKR, Stirrat GM. Chlamydial serology in fertile and infertile women. Lancet **1984**;1:191–193
16. Brunham RC, Maclean IW, Binns B, Peeling RW. *Chlamydia trachomatis:* its role in tubal infertility. J Infect Dis **1985**;152:1275–1282
17. Ånestad G, Lunde O, Moen M, Dalaker K. Infertility and chlamydial infection. Fertil Steril **1987**;48:787–790
18. Quinn PA, Petric M, Barkin M, Butany J, Derzko C, Gysler M, Lie KI, Shewchuck AB, Shuber J, Ryan E, Chipman ML. Prevalence of antibody to *Chlamydia trachomatis* in spontaneous abortion and infertility. Am J Obstet Gynecol **1987**;156:291–296
19. Mårdh PA, Lind I, Svensson L, Weström L, Møller BR. Antibodies to *Chlamydia trachomatis, Mycoplasma hominis* and *Neisseria gonorrhoeae* in sera from patients with acute salpingitis. Br J Vener Dis **1981**;57:125–129
20. Robertson JN, Ward ME, Conway D, Caul EO. Chlamydial and gonococcal antibodies in sera of infertile women with tubal obstruction. J Clin Pathol **1987**;40:377–383
21. Mabey DCW, Ogbaselassie G, Robertson JN, Heckels JE, Ward ME. Tubal infertility in the Gambia: Chlamydial and gonococcal serology in women with tubal occlusion compared with pregnant controls. Bull WHO **1985**;63:1107–1113
22. Plummer F, Laga M, Brunham RC, Piot P, Ronald AR, Bhullar V, Mati JY, Ndinya-Achola JO, Cheang M, Nsanze H. Postpartum upper genital tract infections in Nairobi, Kenya: epidemiology, etiology, and risk factors. J Infect Dis **1987**;156:92–97
23. Hira SK, Hira RS. Congenital syphilis. In: AO Osoba, ed. Sexually transmitted diseases in the tropics. London: Baillière Tindall, **1987**:113–127