SYNDICATION

Associations between *Mycoplasma genitalium*, *Chlamydia trachomatis* and pelvic inflammatory disease

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Objective: To evaluate the association between *Mycoplasma genitalium*, *Chlamydia trachomatis*, and pelvic inflammatory disease (PID)

Methods: A case-control methodology was used. Swab eluates were processed using the QIAamp DNA mini kit. Polymerase chain reaction (PCR) for *M genitalium* was carried out using a real time in-house 16S based assay. An endocervical swab was taken and tested for the presence of *C trachomatis* (ligase chain reaction, Abbott Laboratories), and a high vaginal swab was taken and tested for the presence of *Neisseria gonorrhoeae* and bacterial vaginosis.

Results: Of the PID cases 13% (6/45) had evidence of *M genitalium* infection compared to none of the controls (0/37); 27% (12/45) of the cases had *C trachomatis* infection compared to none of the controls; and 16% (7/45) of cases only had serological evidence of *C trachomatis* infection compared to 5% (2/37) of controls. Cases were more likely to present with *M genitalium* and/or *C trachomatis* than controls ($p<0.001$).

Conclusions: This study indicates that there may be an association between *M genitalium* and PID, and that this relation is largely independent of *C trachomatis*. Future studies need to investigate the pathological basis of the relation between *M genitalium* and PID using samples from women with PID diagnosed using laparoscopy and endometrial biopsy. Little is known about the epidemiology of *M genitalium*: large scale epidemiological investigations are needed to determine the prevalence, incidence, and factors associated with this emerging infection.

Pelvic inflammatory disease (PID) is a key issue facing women's reproductive health and has a multifactorial aetiology. Although *Chlamydia trachomatis* causes a substantial proportion of cases, serological evidence has associated *Mycoplasma genitalium* with PID.[1] Previous investigations have been hampered by the lack of simple, accurate diagnostic methods, but polymerase chain reaction (PCR) assays are now available.[2 3] A recent Kenyan study suggested an association between *M genitalium* and acute endometritis.[4] Here, samples from a case-control study undertaken in England were used to evaluate the relation between *M genitalium*, *C trachomatis*, and PID.

METHODS

A case-control methodology was used which included women aged 16–46 years of age. Patients were derived from a larger case-control study of risk factors associated with PID.[5] A PCR test for *M genitalium* that had been developed by the Public Health Laboratory Service became available halfway through the study, and was included in the protocol. Detailed data on sexual behaviour, demographic factors, and reproductive health were collected as part of the larger PID case-control study, but could not be used here because of the small number of *M genitalium* cases detected and the small overall sample size.

PID cases were diagnosed using the clinical criteria suggested by Hager *et al*—that is, lower abdominal pain, adnexal tenderness, and tenderness with motion of the cervix and uterus.[6] This is the standard definition used in departments of genitourinary medicine. Controls were women attending obstetrics and gynaecology for bilateral tubal ligation, and all underwent laparoscopy as part of this procedure. Two endocervical swabs were taken. One was tested for the presence of *C trachomatis* (ligase chain reaction (LCR), Abbott Laboratories), and the other was tested for the presence of *Neisseria gonorrhoeae*. A high vaginal swab was taken and tested for the presence of bacterial vaginosis (BV). Blood samples were also taken to assess serological evidence of chlamydial infection using the microimmunofluorescence (MIF) test. Results of the MIF test were interpreted as follows: *C trachomatis* titre = 16, no evidence of infection; titre ⩾32, evidence of infection; titre of *C trachomatis* ⩾ titre of *C pneumoniae*, cross reaction unlikely; titre of *C pneumoniae* > titre of *C trachomatis*, indeterminant result.

The *M genitalium* PCR test used the following protocol. A volume of 200 µl of swab eluate was processed using the QIAamp DNA mini kit (Qiagen Ltd) blood and body fluid protocol according to the manufacturer's instructions. For swabs in LCx medium or transport medium 1 µg calf thymus DNA was added as a carrier before extraction. Swabs transported to the laboratory dry were incubated at room temperature in 500 µl phosphate buffered saline for 1 hour and vortex mixed. A volume of 200 µl of expressed fluid was processed using the QIAamp DNA mini kit swab protocol without carrier DNA. Nucleic acid was eluted from QIAamp columns using 50 µl of buffer AE which was incubated on the column for 5 minutes. The same buffer was then used to elute nucleic acid from the column a second time. Amplification was performed by in-house PCR using the LightCycler instrument.

The following primers were used to target the 16S rRNA gene of *M genitalium:* 16SFG2: 5′ CCT TAT CGT TAG TTA CAT TGT TTA A 3′ and 16SRG: 5′ TGA CAT GCG CTT CCA ATA AA 3′.[7] Reactions were set up for the LightCycler consisting of (final concentrations in 10 µl reactions): 500 nM each primer, 200 µM each dNTP, 50 mM TRIS-HCl pH 8.3, 5 mM $MgCl_2$, and 0.65 U Platinum *Taq* polymerase (prediluted in 1 µl 2.5 mg/ml BSA to prevent denaturation). This mix was dispensed and made up to 10 µl using the QIAamp extracts. The LightCycler instrument (Roche Molecular Systems) was programmed as follows: after initial denaturation of 1 minute at 95°C, 50

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Table 1 Evidence of *M genitalium* and *C trachomatis* in cases and controls

	Cases (n=45)	Controls (n=37)
M genitalium	6*	0
C trachomatis (ligase chain reaction with or without serological evidence)	12†‡	0
C trachomatis (serology only)	7	2
Rest	20§	35

*Includes 1 co-infection with *C trachomatis*.
†Includes 1 co-infection with *N gonorrhoeae*.
‡7 had serological evidence of *C trachomatis* infection.
§1 had *N gonorrhoeae*.

cycles were performed consisting of 0 second at 95°C, 0 seconds at 55°C, and 15 seconds at 72°C (program type: melting curves). After each cycle a single fluorescence reading was taken. The results were ascertained using a melt cycle at 0.2°C per second with continuous fluorescence readings. A positive specimen was judged to have a T_m within plus or minus 1°C of the positive control. This was approximately 88.5°C. Positive results were confirmed using a hemi-nested block based PCR.[3 8]

Statistical analysis was undertaken using the Fisher's exact test (STATA 6). Cases and controls were compared in terms of age using the Mann-Whitney test.

RESULTS

A total of 82 women were included in the study, 45 with a clinical diagnosis of PID and 37 from patients undergoing tubal ligation. The median age of the cases was 25 (range 16–43), whereas that of the controls was 34 (range 21–45). Cases were significantly younger than the controls ($p<0.001$).

Evidence of *M genitalium* infection was found in 13% (6/45) of the cases compared to none of the controls, 27% (12/45) of the cases had *C trachomatis* infection (LCR with or without serology) compared to none of the controls, and 16% (7/45) of the cases had only serological evidence of *C trachomatis* infection compared to 5% (2/37) of controls (table 1). BV was not detected in any of the cases and controls. Two cases had co-infections: one with *C trachomatis* and *N gonorrhoeae*, the other between *M genitalium* and *C trachomatis* (this patient also had serological evidence of *C trachomatis* infection). The remaining five *M genitalium* infected patients had no serological evidence of *C trachomatis* infection. Of the patients with *M genitalium* infection, half were over 30 years old. Cases were more likely to present with *M genitalium* and/or *C trachomatis* than controls ($p<0.001$).

DISCUSSION

This is the first case-control study of PID to test for *M genitalium*. The results suggest that there is an association between *M genitalium* and PID, and that *M genitalium* is not merely a commensal organism detected at the site of an STI infection. These findings are similar to those from studies of *M genitalium* in men with non-gonococcal urethritis.[9 10]

There is no standard methodology for sampling the female genital tract for *M genitalium*. Here, the endocervix was used because it was thought to be the site from which *M genitalium* migrates to the upper genital tract. The performance of the LightCycler PCR assay was similar to that of the block based assay using the same primers,[7] but detected fivefold less DNA (equivalent to 10 genome copies) of *M genitalium* in a dilution series. In tests using 28 common micro-organisms, none produced a product with the T_m typical of that from *M genitalium*. However, the testing methodology may lack sensitivity because many of the specimens had previously been tested for *C trachomatis* using the LCR test which includes an incubation step at 95°C in a high magnesium buffer. Anecdotal evidence suggests that mycoplasma DNA may be susceptible to degradation, perhaps due to a relatively low guanine and cytosine content. The inclusion of a serological test would have provided evidence of previous exposure to *M genitalium* but was not carried out because these specialist techniques were not available at the laboratory.

The Hager definition of PID lacks specificity and consequently some patients included in this study may not have had PID. This problem is inherent to all studies of PID. However, the most crucial element in the design of a case-control study is not to include cases in the control group. Here all controls were laparoscoped to ensure that none of the controls had PID.

The multifactorial aetiology of PID is well established but the public health control of PID has largely centred on the control of genital chlamydial infection. *The National Strategy for Sexual Health and HIV* recently published by the Department of Health (England) emphasised the need to screen young women for genital chlamydial infection. Although this is a welcome development, this study is a timely reminder that chlamydial intervention alone is unlikely to eradicate PID. The antibiotics used to treat genital chlamydial infection may be less effective in the treatment of *M genitalium* and, since infection has been shown to be largely independent of *C trachomatis*, it is likely that the epidemiology of these infections may be different.[11]

Effective PID prevention and control rests on improved knowledge of the pathogenesis and epidemiology of the aetiological agents that cause this clinical syndrome. Further studies need to investigate the pathological basis of the relation between *M genitalium* and PID using samples from women with PID diagnosed using laparoscopy and endometrial biopsy. This study was based on an opportunistic cohort and it was not specifically designed to examine the relation between *M genitalium* and PID. A bias within the study was the significant difference in age between the case and control groups. However, since half the patients with *M genitalium* were over the age of 30 years, this source of bias is unlikely to have significantly influenced the study. Nevertheless, results reported here give an interesting insight into the sequelae associated with *M genitalium* and provide directions for future studies. Little is known about the epidemiology of *M genitalium* and specifically designed large scale epidemiological investigations are needed to determine the prevalence, incidence and factors associated with this emerging infection. However, before such studies can be undertaken clear guidelines are needed on the diagnosis of *M genitalium* and the sites that should be sampled.

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Conflict of interest: none declared.

CONTRIBUTORS

IS initiated and designed the project and undertook the analysis with PR; KE and AH developed the *M genitalium* PCR and did the testing; HM undertook the LCR and serological testing; KH, RG, and PH recruited cases and controls for the study.

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ECHO

Quality and use of synovial fluid tests open to question

Please visit the Journal of Clinical Pathology website [www.jclinpath.com] for link to this full article.

The use of synovial fluid tests gives cause for concern, despite their obvious diagnostic value in certain joint diseases, suggests a comprehensive literature review of their applications from the University of Bristol.

The review, based on 300 papers out of an initial trawl of 6556, published between 1980 and 2001, confirms that synovial fluid analysis can be of major diagnostic value. It is especially useful in acute arthritis, when a crystal arthropathy or septic arthritis is suspected, and in intercritical gout.

But standards fall well short of optimal for the traditional assays of microbiology, white blood cell counts, and microscopy for pathogenic crystals. And there are "worrying variations" in reported sensitivity, specificity, and reliability, and scant evidence of quality control, it finds.

Furthermore, the use of the newer cytological and biochemical marker assays is based primarily on anecdotal evidence, with no research into their sensitivity, specificity, and reliability, it says. Added to which, the well known difficulties of interpreting the results of biochemical assays restrict their use in clinical medicine.

The authors point out that the poor standards highlighted by the review may be partly due to the fact that synovial fluid analysis is routinely under researched, excluded from routine diagnostic pathology services, and is a victim of low throughput of samples in most units.

More research is urgently needed, say the authors, who conclude that the immediate priority should be to rationalise the use, and improve the quality control, of synovial fluid assays, given their importance in acute arthritis.

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