The value of *Chlamydia trachomatis* antibody testing as part of routine infertility investigations

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The difference between the two groups was statistically significant (C. trachomatis titres higher than 1 in 32 had tubal damage (35%). When patients present for infertility investigations, it is important to screen this group of patients for chlamydial infection. Serology, using the micro-immunofluorescence technique we assessed the significance of positive serology. There was a marked association between the titre and the likelihood of tubal damage. In the group with low titres (1 in 32) there was only a 5% incidence of tubal damage; however, there was a progressive increase in the incidence of tubal damage in those with higher titres. Twenty out of 57 patients with titres higher than 1 in 32 had tubal damage (35%). The difference between the two groups was statistically significant (P < 0.0001, χ² test). By using *C. trachomatis* antibody testing more widely it may be possible to reduce the number of laparoscopies performed. It should therefore become an integral part of the fertility work-up.

Key words: antibody/Chlamydia trachomatis/hysterosalpingography/laparoscopy/tubal damage

Introduction

Pelvic inflammatory disease (PID) is the single most important cause of tubal pathology leading to infertility. Tubal factors account for between 14–38% of cases of female infertility (Dabekausen et al., 1994). The two organisms most frequently related to upper genital tract infection in the UK are *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, with the incidence of chlamydial genital infection increasing world-wide (Paavonen and Wolner-Hanssen, 1989). *Chlamydia* is now recognized to be associated with at least 50% of cases of acute PID in developed countries and in 50–80% of female patients *C. trachomatis* infection is asymptomatic. Due to the asymptomatic nature of *C. trachomatis*, the diagnosis of tubal disease cannot rely solely on the presence or absence of a history of PID. As the late sequelae of PID (chronic pelvic pain and tubal damage) have major health implications it is important to screen this group of patients for chlamydial infection.

The two most commonly used methods of assessment for tubal disease are still hysterosalpingography (HSG) and laparoscopy (Dabekausen et al., 1994). HSG has been routine in many fertility centres as an initial investigation as it is cheaper and less invasive than laparoscopy. It is sometimes painful, however, and has a low sensitivity (Swart et al., 1995). Laparoscopy is considered the gold standard and has been shown to be better than HSG in tubal assessment, particularly in detecting peritoneal adhesions and endometriosis (Corson, 1977). Laparoscopy is however an invasive procedure and carries with it specific complications. The use of a non-invasive test in conjunction with, or as an alternative to, these would therefore be useful in the initial investigation.

Moore et al. (1982) showed that between 73–79% of infertile women with tubal abnormalities as seen on HSG or direct inspection were positive for *C. trachomatis* antibody (Moore et al., 1982). Serology has since been shown to be more accurate than HSG in predicting the presence of tubal disease (Dabekausen et al., 1994), and when used in conjunction with an HSG it significantly lowers the false-positive rate (Meikle et al., 1994).

When patients present for infertility investigations, *C. trachomatis* infection will have been present for many years. Serological investigation for *C. trachomatis* is more likely to be positive than using antigen detection tests. Traditionally micro-immunofluorescence (MIF) testing has been used to test serologically for chlamydial infection. Depending on how this test is performed, it can be used to differentiate *C. trachomatis, C. pneumoniae* and *C. psittaci* infection but there is also a level of cross-reaction due to shared antigens (Mannion et al., 1991). The MIF test is also technically demanding and labour intensive and as a result is being replaced in some centres by enzyme-linked immunosorbent assay (ELISA) based antibody tests.

The aim of this study was to look at the relationship, if any, between the MIF titre and the degree of tubal damage. The intention was to consider if the MIF titre could be used to determine which patients required a laparoscopy and which patients could have a less invasive procedure, i.e. a HSG or hysterosalpingo-contrast sonography (HyCoSy).

Materials and methods

A retrospective study was carried out looking at all women presenting to the infertility clinic for their first consultation between March 1995 and March 1997. There were 234 new patients during this time, 220 of whom had serology performed. Routine history and physical examinations were carried out on all patients, which included the taking of a high vaginal and endocervical swab for routine bacteriology, *N. gonorrhoeae* and *Chlamydia* antigen testing. Blood was taken for follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin between day 3 and 5 of the menstrual cycle.

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Rubella serology was also checked. A transvaginal ultrasound scan was performed using a Toshiba SSA 250 unit with a 5 MHz probe (Toshiba, Tokyo, Japan).

The serological test used during this period was an indirect MIF technique for C. trachomatis IgG antibodies based on the method previously described (Treharne et al., 1977; Dave Ellis, personal communication). After March 1997 the ELISA technique was used and analysis was therefore not continued.

Tubal patency was tested by HSG or, if there was any indication of pelvic pathology, by laparoscopy and dye test. Tubal disease seen on HSG was defined as obstruction to dye or abnormal dye patterns as defined by the viewer. At laparoscopy it was determined by the presence of adhesions involving the tube, clumping of the tube or obstruction to the dye. All HSG tests were done in the proliferative phase of the cycle with oil-soluble contrast medium. For laparoscopy tubal testing was done with methylene blue dye.

The case sheets were reviewed for those patients with positive titres (i.e. 1 in 32 or greater) with particular reference to parity, previous gynaecological history, symptoms on presentation, past history of PID, swab results (high vaginal and endocervical), transvaginal ultrasound scan findings, results of HSG and/or laparoscopy, fertility treatment received and the outcome of the treatment.

### Results

In 117 of the 220 cases studied (53.2%) the C. trachomatis antibody titre was positive (IgG titre 1 in 32 or higher). Four of these 117 patients had previously had sterilization procedures performed and were therefore excluded from the study. In the group with a titre of 1 in 32 there was a 5% incidence of tubal damage (3/56). There was a significant increase in the incidence of tubal disease in those patients with a titre of 1 in 128 or more. A titre of ≥1 in 128 was found in 26% (57/216) of patients attending the infertility clinic but in this group there was a 35% (20/57) incidence of tubal disease. This difference was statistically significant ($P < 0.0001$, $\chi^2$ test) (Figure 1) when compared to the group with titres of 1 in 32. Table I shows the number of patients with each titre value, the percentage of patients with primary infertility in each group, how the patients were investigated and the percentage with tubal damage. Secondary infertility appeared to be associated with tubal damage (Table II) but in our results there was no significant difference in the incidence of tubal damage in those with titres of 1 in 32 and those with higher values ($P = 0.239$, uncorrected $\chi^2$ test) (Table I).

The method of tubal assessment of these patients according to their C. trachomatis titre is shown in Figure 2. This showed that laparoscopy was performed more often in those groups with high titres. In the group with titres of 1 in 32, 46 HSG and 14 laparoscopies were done but in the group ≥1 in 128, 29 HSG and 31 laparoscopies were done (Table I). All patients with an abnormality apparent on HSG went on to have a laparoscopy. This should have reduced the chance of any abnormality going undetected by the patient only having an HSG.

Despite the high number of cases with positive serology, only one case also had a positive endocervical swab for
C. trachomatis. High vaginal swabs were done in 103 patients and were positive in 14 (13.6%). Six were positive to Gardnerella, three for anaerobes, four for Candida and one swab showed Group B haemolytic Streptococcus.

Transvaginal scan was done in 105 cases, with 36 (34.3%) showing an abnormality. These were polycystic ovaries (n = 19), endometrioma (n = 6), hydrosalpinx (n = 5), fibroid (n = 4) and endometrial polyp (n = 2).

Three patients received prophylactic antibiotics in view of high titre even though there was no evidence of active disease. The use of antibiotics and their value is not known in these patients but it was felt that they may be of value in patients with high titres.

Discussion

Upper genital tract chlamydial infections in women are on the increase and various methods for detecting C. trachomatis infection are available. The presence of C. trachomatis in cervical samples in patients with clinical, laparoscopic or biopsy evidence of PID has not been closely related to upper tract infection, as confirmed in this study (Lee et al., 1995; Wisenfeld et al., 1996). Out of all the patients in our study there was only one positive endocervical swab (<0.5%). Chernesky et al. (1998) showed that only six out of 17 women who had positive cervical swabs had upper genital tract infection and conversely Chlamydia has been cultured from the upper genital tract in patients with abdominal pain and negative cultures (Wöllner-Hanssen et al., 1983). Therefore Chlamydia serology has been thought to be more predictive of upper tract infection and therefore a desirable way to avoid laparoscopy. Our study showed that C. trachomatis titres can be useful in determining which women are at higher risk of tubal disease and that C. trachomatis titres should be done before the method of tubal assessment, i.e. laparoscopy, HSG or HyCoSy, is selected.

Laparoscopy and dye test is considered the gold standard for the evaluation of tubal function but is an invasive and expensive procedure, making it unsuitable for screening purposes. HSG is a less invasive test but is of limited use for detecting tubal patency because of its low sensitivity, although its high specificity makes it a useful test in confirming the presence of tubal obstruction (Swart et al., 1995). Its high false-positive outcome is thought to result from tubal spasm, dissimilar tubal filling pressure, too high viscosity of the contrast medium and faulty technique (Dabekausen et al., 1994). When HSG is combined with C. trachomatis titres, the false-positive rate is significantly lowered (Meikle et al., 1994). HyCoSy has a similar accuracy in detecting the tubal patency as HSG, although it has other benefits (e.g. better visualization of the uterine cavity, no radiation exposure) which may make it a more popular test in the future (Reis et al., 1998). A problem with HSG is that Chlamydia causes adnexal adhesions as well as tubal obstruction and these are best picked up by laparoscopy (Swart et al., 1995). Adnexal adhesions are much more common in women with positive Chlamydia titres (Walters et al., 1988; Tanikawa et al., 1996) showing that those women with high titres should therefore have a laparoscopy.

C. trachomatis antibody testing in infertility investigations

High titre of chlamydial IgG antibody are associated with inflammatory tubal damage, pelvic adhesions and increased risk of tubal pregnancy (Sheffield et al., 1993; Land et al., 1998). The presence of peritubular adhesions may also limit tubal motility and interfere with ovum capture (Tanikawa et al., 1996). These findings are in keeping with the results of this study and show the usefulness of C. trachomatis antibody testing as a routine baseline investigation in the infertility clinic.

Chlamydia antibody titres cannot be used as the sole test of tubal patency. Patients may have an unrelated cause for adhesions (e.g. endometriosis or salpingitis due to another micro-organism). Also some patients who have had previous C. trachomatis infection have no detectable antibody (Puolakkainen et al., 1986). These authors also showed that the sensitivity of the antibody test is critical as IgG titres can decrease over time. During a follow-up period of 3–6 years there was a significant decline in IgG titres in 26 (43%) patients. Chaim et al. (1992) noted that in a 5 year interval antibody titres fell in 18 out of 25 patients but no patient became seronegative. False positive results can occur because of cross-reactivity in MIF tests due to antibodies to C. pneumoniae (Moss et al., 1993), which is widely prevalent in Europe and the USA (Mol et al., 1997).

Chlamydia infection can be detected by a variety of methods. No single test has total diagnostic accuracy. The MIF test, which was used in this study, has been evaluated extensively in the investigation of tubal factor infertility (Jones et al., 1982) and ectopic pregnancy (Chow et al., 1990). The whole-inclusion immunofluorescence (WIF) test, which has limited published evaluation, and the ELISA are used but there has also been sharp focus on the heat shock protein-60 (HSP-60) test. Reaction to the 60 kDa antigen of C. trachomatis infection, a HSP analogue, has been suggested as a possible marker for the development of chronic sequelae after C. trachomatis infection. Several studies have shown this test to have a high sensitivity and high specificity (Wagar et al., 1990; Toye et al., 1993) and anti-HSP antibody rates in patients with complete tubal occlusion have been shown to be significantly higher than in those patients with normal Fallopian tubes (Freidank et al., 1995). Chernesky et al. (1998) compared results of antibody assays with cervical culture and C. trachomatis plasmid DNA by polymerase chain reaction (PCR) on endometrial biopsies. They compared a combination of MIF, WIF, ELISA and HSP-60. No single serological assay combined 100% sensitivity and specificity in the study, although the HSP-60 enzyme immunoassay showed 100% specificity and 42.9% sensitivity. Routine availability of this test could perhaps further reduce the number of laparoscopies performed in our study group. Cervical IgG antibodies to C. trachomatis have also been shown to be strongly related to chlamydial infection (Witkin et al., 1997) and may well prove to be a reliable method of detection.

In summary, our study showed a significant increase in the degree of tubal damage in women with C. trachomatis MIF titres ≥1 in 128. The higher the titre the more likely there would be tubal damage. In this study we only looked at those with positive serology to determine a cut-off level, although there will be a proportion of patients with negative titres who
have tubal damage due to other causes (e.g. endometriosis). It has already been mentioned that seropositive patients do not seem to become seronegative (Puolakkainen et al., 1986), making chlamydial damage very unlikely in this group. Previous work has shown that combination of HSG and C. trachomatis antibody titres will give a false negative rate of approximately 5% (Meikle et al., 1994), and therefore are best used in those patients with a low titre (<1 in 128). In patients with a higher titre, a laparoscopy would be the better procedure as there is a significantly higher incidence of tubal disease. In our setting this would mean that a quarter (26.3%, 57/216) of new patients would initially have a laparoscopy based on their initial titre. Although other patients would undergo a laparoscopy for other reasons (e.g. assessment of endometriosis), the majority would undergo a less invasive investigation (HSG or HyCoSy). This treatment also has beneficial cost implications. The choice of method for tubal assessment should therefore be taken after the results of the C. trachomatis antibody titres are known.

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References


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