CHAPTER IV

DISCUSSION

In recent years, C. trachomatis has been known as one of the most important sexually transmitted agents (7, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 131, 132, 134) and it becomes the most prevalent cause of non-specific urethritis (NSU) in Thailand (131). Definite diagnosis of C. trachomatis infections is generally made after cultivation in cell culture. In the present study, C. trachomatis was isolated in 69 of 200 NSU patients (34.5%). This incidence was consistent with the results of Schachter (81) and Hagay et al (134) who reported that urethral specimen from 30-60% of patients with NSU yield C. trachomatis The prevalence of C. trachomatis cultivation from urethral specimen depends on the method of specimen collection, the use of non-toxic endourethral swabs and the sensitivity of the isolation procedure which is normally less than 100% (134). Certain disadvantages of this technique exists including time consumption, expenses, performance difficulty and requirement of special laboratory equipment and trained personnel. Consequently, chlamydial antibody was used as an adjunct to cell culture in diagnosis of chlamydial infection and is useful for epidemiologic surveys (29, 33, 47, 122, 132). Of the 200 sera examined in this study specific IgG, IgM and IgA chlamydial antibodies were present in 173 (86.5%), 17 (8.5%) and 10 (5%) respectively by m-IF and 186 (93.0%), 2 (1.0%) and 10 (5.0%) respectively by IF. Nevertheless, only 36-37.5% of these patients with IgG antibodies demonstrated C. trachomatis upon culture. It is conceivable that IgG antibodies may persist for years and thus could not be used to indicate current chlamydial infection. This is in accordance with

Darougar who reported that serum antibodies have little value in the definitive diagnosis of chlamydial infection and cannot replace isolation method (47). The detectability of IgM and IgA antibodies were very low. As for antibody in secretions, IgG, IgM and IgA antibodies were found in 54 (27.0%), 3 (1.5%) and 58 (29.0%) respectively by m-IF and 47 (23.5%) for IgG antibody, 53 (26.5%) for IgA antibody by IP. No secretory IgM antibody was detectable by IP test in this study. The low incidence of IgM antibody both in serum and secretion may be due to the fact that IgM antibody can be found in primary infection but rare in secondary or chronic infection (47,74). Moreover, this antibody lasts for approximately one month following infection (135). In this study most patients had previously suffered from the disease so it may be due to chronic infection. The IP method was slightly more sensitive than m-IF for serum IgG detection but was less sensitive for detection of IgG and IgA antibodies in secretion. It appeared to be of little value for IgM antibody detection both in serum and in secretion. Only 2 of the 17 serum IgM antibody found by m-IF were positive by IP and none of the 3 IgM antibody in secretion detectable by m-IF were positive by IP. This was supported by Fin et al, 1983 who demonstrated that IgM antibody test by enzyme-linked immunosorbent (ELISA) was not as good as IgG antibody detection. Thus ELISA-IgM is less specific and less sensitive than m-IF (2, 132). Avidin-biotin IP was utilized to increase the detectability of secretory IgA to 84 (42%)

Of the 69 culture positive patients, there were 4 without detectable chlamydial since patients' record revealed that urethral secretions were collected only ten days or less after disease contact. Antibodies may develop later on as it has been reported by Yoshizawa et al. in 1987 that in somme cases serum IgG or IgA chlamydial antibodies were detected after two weeks or more following infection (135).

The serum chlamydial IgG antibody titers by m-IF and IP as shown in Figure 12 demonstrated that the IgG antibody titers obtained by the two methods correlated well with a correlation coefficient (r) of 0.97. This is in agreement with Cevenini et al who utilized both methods to study specific IgG antibody to C. trachomatis in non-gonococcal urethritis patients with a correlation coefficient of 0.92 (56). Haikin et al who studied IgG antibody to Varicella-Zoster Virus-Induced Membrane using these two methods also found good correlation (55). Moreover, Yoshizawa et al in 1987 revealed that both IgG and IgA chlamydial antibody titer obtained by the two methods correlated well with each other with correlation coefficients of 0.8 each (135). The IP is also highly sensitive with respectable positive and negative predictive values comparing with the standard m-IF method. This agreed with Haikin et al and Cevenini who had reported that the IP technique was as sensitive as the m-IF technique within 2-fold dilution (55, 56). The specificity of IP was rather low in our study, this might be attributed to the data shown in table 9 that of the 27 m-IF negative patients, although 15 were positive by IP, 11 had only one dilution difference from m-IF. This is in accord with Bialasiewiez and Jahr who reported in 1987 using IP for detection of IgG chlamydial antibody in conjunctivitis patients that this test was sensitive nevertheless not specific. (29). When the positive IP that was determined at titer $\geq$ 1:16 the sensitivity was decreased to 85.6% whereas the specificity was increased to 85.2%.

When the results of chlamydial antibodies detection in secretions by IP method was compared with m-IF (table 11), the specificity, positive and negative predictive values were high with good sensitivity for IgG antibody. As for secretory IgA antibody, the IP produced fair sensitivity (79.31%) which was much improved when avidin-biotin IP was used (94.83%). Thus it is

obviously shown that IP test can become an alternative method for the detection of chlamydial IgG or IgA antibody owing to its high sensitivity, specificity, positive predictive and negative predictive values. The method is also simple, rapid, inexpensive and requires only a light microscope whereas the m-IF is more costly, technically difficult and requires well-trained personnel and a fluorescent microscope. In addition, the IP antigen slides can be stored at -20°C or -70°C for at least 6 months.

In view of the application of antibody detection in secretions, several scientists have documented on the close relationship of IgA antibody to chlamydial infection (37, 47, 58, 106). According to Terho and Meurman, absence of local IgA antibody was a strong indication against actual chlamydial infection (37). This was supported by Darougar who provided evidence suggesting that the presence of specific IgG or IgA antibody at any level in local discharges were useful indicators for the provisional diagnosis of infection (47). In the present study, the presence of IgA or IgG chlamydial antibody in secretion correlate with isolation of C. trachomatis with the detection of secretory IgA antibody by avidin-biotin IP appearing to be the best method ($X^2 = 61.50$; $P < 0.01$) (Table 21,22 appendix VI and Table 13). Consequently, this method was further evaluated for its applicability to detect current chlamydial infections. In our study, definite diagnosis of chlamydial infection is indicated by a positive isolation of C. trachomatis from urethral specimen and a possible diagnosis was set by the demonstration of secretory IgA chlamydial antibody in urethral secretion by the standard m-IF methods. Combination of these two criteria should provide for a fairly accurate estimation of current chlamydial infection.

Based on the definite diagnosis alone as golden standard, secretory IgA by avidin-biotin IP could detect infection with a sensitivity of 79.71% with high negative predictive value of 87.93% (Table 13). The 14 cases with no detectable secretory IgA but poss C. trachomatis upon isolation may be ascribed to early infection. As for the 29 cases with IgA antibody in secretion without detectable organism upon isolation, it was possible that the chlamydia being neutralized by the antibodies did not grow well in vitro as rationized in previous reports (5,58,59). Direct immunofluorescence would be helpful to verify this in these specimens. When the two combined diagnosises were used as golden standard the sensitivity, specificity and positive predictive value of avidin-biotin IgA test were increased to 81.84%, 87.72% and 83.33% respectively. The 14 cases with neither chlamydia upon isolation nor secretory IgA by m-IF yet positive avidin-biotin secretory IgA may be attributed to the higher sensitivity of avidin-biotin IP over the standard method, isolation. Using more sensitive method such as direct immunofluorescence instead of isolation may improve this result.This indicates that the avidin-biotin IP can be used as an alternative method for chlamydial isolation and m-IF to help diagnosing chlamydial infection.

Research advantages

1. To provide an appropriate test which is simple, rapid and highly efficient to detect C. trachomatis infection in non-specific urethritis (NSU) patients.

2. To realize the situation of C. trachomatis infection in NSU patients in Thailand which may be very useful for planning on prevention and control of the disease.

3. To acquire a guideline of rapid immunoperoxidase for other chlamydial diseases.

Further study and Suggestion

The secretory IgA antibody by avidin-biotin IP should be investigated in normal control or healthy male in order to confirm the specificity of IgA antibody present in chlamydial infection.

Moreover, detection of IgG or IgM in secretions by avidin-biotin should be tried and evaluated for their significance in diagnosing chlamydial infection.

It is also very interesting to use this rapid immunoperoxidase test to detect specific chlamydial antibodies in other diseases caused by this agent. For instance, salpingitis, pelvic inflamatory, infertility and conjuctivitis both in sera and local secretions. Especially in neonatal infection such as conjuctivitis and pneumonia in infants getting the organism from their infected mother's birth canal are very serious problems nowadays. Investigation of specific antibodies using rapid immunoperoxidase in these patients may be very helpful in the diagnosis of the diseases.

CONCLUSIONS

1. Titers of serum IgG antibody as detected by IP were comparable to that found by m-IF ($r = 0.97$).
2. The IP method was as sensitive as m-IF for the detection of serum IgG antibody
3. The IP method produced good results for detection of IgG and IgA antibodies in secretions in comparison with m-IF, especially when avidin-biotin was used to detect IgA antibody in secretion.
4. The IP seemes not to be successful for IgM antibody detection both in serum and secretion.
5. Avidin-biotin IP for IgA antibody in secretion can be used to detect chlamydial infection with the sensitivity of 81.40% and specticity of 87.72%.
6. Of the 200 male patients with NSU, 69 (34.5%) yield C. trachomatis upon cultivation and 86 (43%) were possibly afflicted with current C. trachomatis infection.