CHLAMYDIA TRACHOMATIS ANTIGEN IN FEMALE GENITAL TRACT INFECTION

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Thirty cases of female genital tract infection were investigated for the presence of *Chlamydia trachomatis* antigen. Endocervical swabs obtained were subjected to antigen detection by enzyme immunoassay. Rabbit antiserum to chlamydial lipopolysaccharide was used in a card test. Anti rabbit immunoglobulin G conjugated to alkaline phosphatase with a chromogenic substrate 5 bromo-4 chloro-3-indolyl phosphate and nitro blue tetrazolium were used for the enzymatic reaction. Chlamydial antigen could be detected in four out of thirty samples (13.3%). In contrast direct immunofluorescence detected 5 cases (16.6%). Although less sensitive, enzyme immunoassay can be used as a rapid diagnostic tool in detecting *Chlamydia trachomatis* antigen in genital infections.

Key Words : Chlamydia trachomatis, Antigen, Female genital tract

Introduction

Infections caused by *Chlamydia trachomatis* continue to be a public health problem. Trachoma, sexually transmitted infections and neonatal pneumonia are caused by chlamydia. Rapid diagnosis of chlamydial infections is essential for instituting proper treatment of individual patients and contact tracing. Standard conventional culture techniques are expensive and time consuming and not within the reach of the routine laboratories in developing countries. Detection of chlamydial antigens by a number of rapid tests has come into picture.[1-4] These methods are less sensitive compared to culture but cost effective especially for screening populations at moderate risk.

Direct detection assays are being increasingly used on cervical specimens for *Chlamydia trachomatis* antigens in lieu of conventional cell culture techniques.[4-8, 10] Enzyme-immunoassay (EIA) and direct fluorescent antibody (DFA) assays are less sensitive and specific and results are available considerably earlier than those from cell culture which is the "gold standard".[10] This study compares the detection of *Chlamydia trachomatis* antigen by using Enzyme Linked Immunosorbent Assay (ELISA) in a card test "Immunocomb" with the fluorescent labelled monoclonal antibody test (DFA) using "Imagen" test kit on cervical specimens of symptomatic patients.

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Materials and Methods

Patients : Endocervical swabs were obtained from thirty patients attending the out patient department of Obstetrics and Gynaecology, JIPMER hospital, Pondicherry. All patients included in the study group were symptomatic. Most patients had a history of white discharge, itching, burning sensation in the external genitals. The age range was between 22 to 37 years (mean 29.4 years).

Specimen collection and processing : Endocervical specimens were collected from patients with history of discharge using 3 swabs supplied by Orgenics Ltd. A preliminary swab was used to remove endocervical mucus prior to sampling and after that sampling swabs (two) were inserted to endocervix and

rotated for 15-30 seconds without touching the vaginal wall. One sampling swab was put into a tube having storage reagent for inhibition of microbial growth. The specimens were stored at -70°C till used. With the second sampling swab smears were prepared (6 mm diameter) wel area, air dried and fixed in fresh acetone for 5 minutes, The slides were further air dried and kept at -20°C for not more than one week before testing.

Test proper : Immunocomb card assay was carried out as per the instructions supplied by the manufacturer enclosed in the kit. Briefly, chlamydial antigen was extracted from the sampling swab by heating. Rabbit antiserum to chlamydial lipopolysaccharide was used in the card test. Antirabbit immunoglobulin G conjugated to alkaline phosphatase with a chromogenic substrate 5-bromo-4 chloro-3 indolyl phosphate and nitro blue tetrazolium were used for the enzymatic reaction. Results were read as per the manufacturers instructions.

Imagen direct fluorescent antibody assay is a qualitative test for the detection of *Chlamydia* in specimens, the test reagent contains fluorescent isothyocyanate (FITC) conjugated monoclonal antibodies. The genus specific monoclonal antibody will detect elementary bodies from all human serovars of *Chlamydia*. Briefly, the conjugated antibody is used in a one step direct immunofluorescence technique. Specimen slides were incubated with the FITC conjugated reagent for 15 minutes, excess reagent removed by washing with PBS, stained areas were viewed using NIKON Optiphot 2 fluorescence microscope with filter system for FITC. Elementary bodies were seen as bright apple green fluorescence against background counter stained material. Positive control was run each time.

Results

Chlamydial antigens/elementary bodies were detected using enzyme immunoassay and direct immunofluorescence test. Four cervical specimens were positive for Chlamydial antigens out of 30 samples detected by enzyme immunoassay, whereas 5 samples tested positive by direct immunofluorescence test. One specimen was positive by DFA and tested negative by ELISA whereas one other specimen was positive by ELISA and negative by DFA.

Discussion

In the present study the performance of the two antigen detecting systems, an enzyme immunoassay Immunocomb card test and a direct fluorescent antibody test Imagen using FITC labelled genus specific monoclonal antibodies, were compared. Five out of 30 samples were positive (16.6%) by DFA, 4 tested positive by ELISA showing higher degree of sensitivity with DFA. As the test has to be performed on the same specimen, two specimens had to be collected which might have diluted the infectious material resulting in low sensitivity with ELISA. However antigen detection methods have a lower sensitivity than cell culture.[2,4] As suggested by Olafur et al,[11] lower sensitivity may be attributed to dilution factor and in their study EIA was most sensitive assay for antigen detection on urethral specimens. Although lacking sensitivity, ELISA may be used as an alternative to DFA, which is cost prohibitive, requires expensive equipment, trained personnel and is subjective giving high degree of false positivity.

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