Original
article*Chlamydia trachomatis* in hydrocele fluid

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Objective: To determine the presence of *Chlamydia trachomatis* in hydrocele fluid.**Methods:** 90 male patients with hydrocele of tunica vaginalis from an endemic area for bancroftian filariasis were investigated for the presence of *Chlamydia trachomatis* in their hydrocele fluids. *C trachomatis* antigen detection tests—a direct immunofluorescence assay and an enzyme immunoassay along with polymerase chain reaction assay for amplification of a 517 bp fragment of *C trachomatis* endogenous plasmid—were used in this study. The patients were also tested for the presence of microfilaria in their hydrocele fluids and night blood. Histopathological examination was carried out to detect adult filarial worm in tunica vaginalis testes.**Results:** Eight (8.88%) patients had chlamydia antigen in the hydrocele fluids; *C trachomatis* plasmid sequences could be amplified from five of these. Seven (7.77%) patients had microfilaria in the hydrocele fluids, three of them having adult worm in tunica vaginalis.**Conclusion:** *C trachomatis* infection might be associated with hydrocele in some of these patients.

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Keywords: hydrocele; *Chlamydia trachomatis*; filariasis**Introduction***Chlamydia trachomatis* is emerging as a major cause of sexually transmitted genital tract infections in both developing and developed countries.^{1,2} As a consequence of chronic inflammation and cicatrization produced by the chlamydial infection in the genital tract, it has been implicated extensively in female infertility, mainly tubal and ectopic pregnancy.³ It has also been implicated in epididymitis.⁴Hydrocele is a common condition seen in coastal areas of India and other parts of the world.⁵ Although filariasis is reported to be the most common cause of hydrocele,⁶ in more than 50% of the cases of hydrocele the aetiology remains unestablished.⁷ It is possible that, owing to chronic inflammation and cicatrization, chlamydia may be responsible for hydrocele in some cases.In this study we investigated 90 cases of hydrocele for the presence of *C trachomatis* in hydrocele fluids by antigen detection assays and a polymerase chain reaction assay (PCR) in addition to investigations for the presence of filarial agent.**Materials and methods**Hydrocele fluid was collected from 90 married male patients aged 25–50 years undergoing surgery at Jawaharlal Institute of Post Graduate Medical Education and Research Hospital, Pondicherry, India. No patient gave any past history of urethritis or epididymitis. Approximately 600 µl of hydrocele fluid from each patient was received in dry ice in our laboratory at New Delhi for antigen detection tests and PCR for *Chlamydia trachomatis*.

A part of the hydrocele fluid from each patient was examined under a light microscope for the presence of microfilaria on the

day of collection. A part of the tunica vaginalis of the testes was collected from each patient and examined histopathologically for presence of the filarial adult worm at the institute of collection.

The night blood was collected from each patient and was examined under a light microscope for presence of microfilaria at the Vector Control Research Centre, Pondicherry.

C TRACHOMATIS ANTIGEN DETECTION IN THE HYDROCELE FLUIDS The hydrocele fluids were tested for presence of *C trachomatis* antigen by direct immunofluorescence assay (Syva Microtrak, UK) and a monoclonal based enzyme immunoassay (EIA).⁸**Direct immunofluorescence assay***C trachomatis* Direct Specimen Test kit was used for this purpose. The test was performed according to the manufacturer's instructions. Briefly, 100 µl of the hydrocele fluids were cytocentrifuged on to the wells of Teflon coated slides (Blue Star, India) with a cytocentrifuge (Shandon, UK). After air drying the slides were fixed in methanol for 10 minutes. The smears were stained with 30 µl of the FITC conjugated *C trachomatis* murine monoclonal antibodies and incubated at room temperature for 15 minutes. The slides were washed in distilled water, air dried, mounted, and observed under ×100 objective of a fluorescent microscope (Nikon, Japan). A positive and negative control (provided with the kit) were examined with each batch of the test. In the direct immunofluorescence assay, the chlamydia particles were seen as bright, green, regular, spherical, fluorescing particles. Any specimen showing a minimum number of 10 such particles was taken as positive.Department of
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Enzyme immunoassay for antigen detection

The ELISA assay was carried out as described earlier.⁸ Briefly, wells of an ELISA plate (Nunc, Denmark) were coated with 200 ng of purified rabbit antibodies against *C trachomatis*. After blocking the wells with 3% bovine serum albumin (Sigma Chemicals, USA) and 2% skimmed milk powder, 100 µl of the hydrocele fluid was added. The plates were incubated at 37°C for 1 hour. After thorough washing, 100 µl of a monoclonal antibody⁹ at 1:200 dilution was added. After further incubation and washing, antimouse IgG conjugated with peroxidase (Dakopatt, Denmark) was added. Following incubation and washing, the substrate (Orthophenylenediamine Sigma) was added and kept till colour development. The reaction was stopped with 2 N H₂SO₄. The reading of optical density (OD) values were taken with an ELISA reader (Multiskan Plus, UK). Negative and positive controls (cervical swab specimens positive for chlamydia antigen) were included in each batch. In the enzyme immunoassay, average of OD values obtained from the mean value of two positive controls and mean value of two negative controls were taken as the cut off value. Any clinical specimen giving a higher value than the cut off value was taken as positive.

PCR ASSAY FOR DETECTION OF *C TRACHOMATIS* IN HYDROCELE FLUIDS

The PCR assay was performed in the hydrocele fluids for amplification and detection of the 517 bp sequence region of *C trachomatis* endogenous plasmid.¹⁰

Briefly, the DNA was extracted from 200 µl of the hydrocele fluids by adding equal amount of lysis buffer containing 50 mM TRIS HCl pH 8.0, 50 mM KCl, 2.5 mM MgCl₂, 0.45% Tween 20, 0.45% NP 40, and 200 µl of proteinase K (Gibco, BRL, MA, USA) in proteinase K buffer. The mixture was incubated at 56°C for 3 hours followed by phenol chloroform extraction procedure. The DNA containing supernatant was carefully collected and the residual contaminating protein was removed with 7.5 M ammonium acetate precipitation. The DNA was ethanol precipitated, dried, and stored till the PCR assay was carried out.

The amplification of the plasmid DNA was carried out in a 50 µl reaction mixture containing 4.5 mM MgCl₂, 0.2 mM d NTPs, 25 pmol each of the sense and antisense primers, 1.25 units of thermostable Taq DNA polymerase (Gibco BRL, MA, USA), 5 µl of 10X reaction buffer containing 10 mM TRIS HCl pH 8.3, and 50 mM KCl. To this reaction mixture was added 5 µl of denatured extracted DNA. The amplification was carried out in a DNA thermocycler (Technee, Cambridge).

The amplified product was subjected to electrophoresis in a 1.5% agarose gel along with a 100 bp ladder (Gibco, BRL) as molecular weight marker. The gel was observed under a ultraviolet transilluminator (UVP, USA) for the amplified product.

Results

Seven of the 90 patients studied had microfilaria in the hydrocele fluids, one of these was, in addition, positive for *C trachomatis* antigen. Three of these patients had fragments of filarial adult worm in tunica vaginalis on histopathology. These three patients were negative for chlamydial infection. None of the patients had microfilaria in the night blood. Thirty two of the 90 (35.55%) patients had history of extramarital sexual contact.

Eight of the 90 hydrocele fluids tested were found positive for chlamydia antigen in the direct immunofluorescence assay. The same eight specimens were found positive for chlamydia antigen in the enzyme immunoassay. From these eight patients four had history of extramarital sexual contact.

In the PCR assay, the 537 bp region of the *C trachomatis* endogenous plasmid could be amplified from five of the hydrocele fluid specimens. All five of these specimens were positive for *C trachomatis* antigen by both the antigen detection assays and one of them was positive for microfilaria.

Discussion

Hydrocele is one of the predominant manifestations of filariasis in males, particularly in areas endemic for periodic bancroftian filariasis in the Indian subcontinent, South East Asia, Africa, and Latin America.¹¹ However, in a maximum of 45% cases of hydrocele, conclusive evidence of filariasis can be demonstrated.^{7, 12} In the present study, conclusive evidence of filariasis was demonstrable only in seven (7.77%) patients, as they had microfilaria in the hydrocele fluid and three had adult filarial worm in tunica vaginalis testes. Serological tests for filarial antibody detection were not done in these patients since they were from a hyperendemic area for filariasis; therefore, most of them would have possessed filarial antibodies from repeated past exposures. In a previous study from a highly endemic area in India for filariasis, filarial infection could be demonstrated as a definitive cause of hydrocele in only 43% of the cases.⁷ Similar findings has been reported from Puerto Rico.¹³

Therefore, there may exist other infective causes which, when superimposed on filarial infection, can lead to hydrocele. In women, the most important complications of chlamydial genital tract infections are tubal infertility and ectopic pregnancy as consequences of salpingitis and subsequent fibrosis.² In males, chlamydia infection is a documented cause of epididymitis.⁴ Although not well studied, some of these cases may progress to cicatrization and fibrosis as occurs in ocular infections and the female genital tract, leading to hydrocele. In experimental studies, subsequent to *C trachomatis* inoculation into the vas deferens, epididymitis was observed in all animals studied clinically and histologically.¹⁴ However it must be noted that chlamydial infection is prevalent worldwide whereas hydrocele is seen in areas endemic for filariasis. The high endemic zone of filariasis is also an underdeveloped area with

low genital hygiene. A large number of patients in this study group (35.55%) had a history of extramarital sexual contact; therefore, infections like those caused by chlamydia can lead to epididymitis and possibly precipitate and/or accentuate the fluid collection in the hydrocele sac.

In the present study more patients, eight (8.88%), had *C trachomatis* antigen in their hydrocele fluids than microfilaria, which was present in seven (7.77%) of them. Although the difference was not significant ($p = > 0.5$), the laboratory evidence for *C trachomatis* infection was at least as apparent as was filarial involvement in this group of hydrocele patients. Moreover, one of the patients had both microfilaria and chlamydia antigen in the hydrocele fluid, suggesting a dual involvement of both the organisms. This may simply be explained by the burnt out filarial infection where it is difficult to identify the organism.

In this study, Syva Microtrak direct immunofluorescence assay and a monoclonal based enzyme immunoassay⁸ were used for chlamydia antigen detection. In past studies, the direct immunofluorescence assays for chlamydia antigen detection using Syva Microtrak test kit were found to be 96.3–100%^{15 16} sensitive and 96–99.5%^{15 16} specific compared with isolation of chlamydia from clinical specimens in tissue culture; said to be the “gold standard” of laboratory diagnosis for chlamydial infection.¹⁷ The Syva Micro Trak test kit has been used as the reference method by reputable laboratories.

The enzyme immunoassay used for antigen detection in this study was a highly sensitive and specific antigen detection method, comparable with the Syva kit.⁷ The same specimens were positive for chlamydia antigen by both of the assays.

In the polymerase chain reaction assay, chlamydia plasmid sequences could not be amplified from three of the specimens giving positive result in the antigen detection assays. There is a possibility that this was due to the presence of inhibitors in the hydrocele fluids.¹⁸ As the total quantity of hydrocele fluids available was limited, the tests could not be repeated. Serological tests for *C trachomatis* antibody detection were not conducted in these patients as antibody detection is gener-

ally not helpful in the diagnosis of current chlamydial infections.¹⁷

From this study it is evident that *C trachomatis* may be implicated as an agent associated with hydrocele either alone or in combination with other infectious agents mainly filarial infections. Further large scale studies are necessary to determine the exact extent of chlamydial involvement in hydrocele.

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