

Evaluation of *Trichomonas Vaginalis* Isolates from Symptomatic and Asymptomatic Patients in Mouse Model

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Abstract

Trichomoniasis, a worldwide prevalent infection, is a perfect example of interplay between the infecting parasite and the host, on which the presentation of disease depends. To study the pathogenesis, animal model is required for establishment of infection. In this study, by using strains of *Trichomonas vaginalis* isolated from vaginal swabs and/or urine samples and maintained in axenic form from 10 symptomatic and 10 asymptomatic female patients, the infections in BALB/c mice have been evaluated. Based on parasitic load, experimental peak infection in vagina of estradiol and *L. acidophilus* treated female BALB/c mice was observable on 5th post infection day. This was significantly higher in mice infected with isolates from symptomatic patients in comparison to the other group. Gradual increase up to day 5 followed by decline in parasites, polymorphs and vaginal epithelial cells was observed in mice infected with isolates from symptomatic subjects in contrast to mice infected with asymptomatic patients' isolates or control animals at all time intervals.

Keywords: Trichomoniasis vaginalis, Animal model, Lactobacilli, Iran

Introduction

Trichomoniasis, the disease of human urogenital tract, is one of the commonest sexually transmitted disease in the world with an estimated of twelve million new cases reported to the Centre of Disease Control (the USA) every year (1). Human trichomoniasis has gained more attention recently as it is thought to predispose to human immunodeficiency (HIV) infection (2) and cause abnormal outcome of pregnancy (3). The mechanism associated with pathogenesis of this disease is not clearly understood. An in vivo model of *T. vaginalis* infection could provide insight into the pathophysiology and immunology of this disease. Several attempts at establishing *T. vaginalis* infection in mammals have been made with monkey, guinea pigs, hamsters, rats and mice (4). Previous attempts

at intravaginal growth determined that pretreatment of animals with estrogens is essential for establishing infection (5). Major differences exist between the genital flora of women and that of mice. The most obvious of these differences involves *Lactobacillus* species. Although *L. acidophilus* is dominant in a healthy woman's vagina, recent work has shown that Lactobacilli are harbored by only a small percentage of mice (6). However concentration of *L. acidophilus* has been reported that reduced in the presence of *T. vaginalis* infection (7).

The minimal amount of lactobacilli and the neutral pH documented for animals (8), suggested that high number of lactobacilli and low pH are characteristic of the human vagina. In this report we evaluated the infection in mice with strains isolated from symptomatic and asymptomatic patients. Recent work with a

modified intravaginal mouse model of *T. vaginalis* infection has shown that the addition of *L. acidophilus* prior to inoculation with *T. vaginalis* resulted in a significantly more consistent and sustained *T. vaginalis* infection compared to a control group of mice that were not pretreated with *L. acidophilus* and the addition of *L. acidophilus* did not significantly alter the resident mouse vaginal flora (4).

Therefore the aim of the present study was to evaluate the parasite load in experimentally infected animals with isolates from women with or without symptoms.

Materials and Methods

Organisms Clinical samples (500) were collected from the department of Obstetrics and Gynecology, PGIMER, Chandigarh India. Isolates obtained from patients complaining of vaginal discharge and/or pruritis, dysuria, and dyspareunia were considered as symptomatic patients isolates. Isolates obtained from patients attending the clinic for routine checkup, infertility or some other gynaecological problems with no complain of vaginal discharge and/or pruritis, dysuria and dyspareunia were considered as asymptomatic patients isolates.

Midstream urine samples and two sterile cotton vaginal swabs were employed for wet smear examination and culture according to the method described previously (9). Twenty local *T. vaginalis* isolates from symptomatic and asymptomatic patients, axenically cultivated in modified Diamonds TYI-S-33 medium, pH 6.2 (10), supplemented with 10% heat inactivated horse serum, 100 U/ml penicillin and 100 µg/mg streptomycin and every 2-3 days subcultured.

Lactobacillus acidophilus *L. acidophilus* strain (ATCC 4356) was obtained from Institute of Microbial Technology (IMTech, Chandigarh, India) and maintained in screw capped tubes containing *Lactobacillus* Man, Rogosa Sharp (MRS) broth overnight at 37°C in 5% CO₂. *L. acidophilus* was harvested by centrifugation at 500×g for 10 min and washed thrice in PBS

(pH 7.2). Final pellet was resuspended in PBS and adjusted to 2×10⁶ cells/ml (11).

Induction and establishment of infection in mice

Female BALB/c mice, 4-6 weeks old, were used for *In vivo* studies. Animals were divided into three groups as follows:

Control group consisted of 8 normal mice with estradiol valerate treatment and inoculated with *L. acidophilus*. Symptomatic group consisted of 8 mice, which was pretreated with estradiol valerate and inoculated with *L. acidophilus*. Each group was infected with clinical isolates of *T. vaginalis* from symptomatic patients. Asymptomatic group included of 8 mice, which was pretreated with estradiol valerate and inoculated with *L. acidophilus*.

Each group was infected with a strain of *T. vaginalis* isolated from asymptomatic patients *T. vaginalis*, 10 symptomatic isolates and 10 asymptomatic isolates were used for experimental induction of infection in mice. Experimental infection was induced according to the previous method (12). Briefly, on day 0, mice (8 per group) were injected subcutaneously in the flanks with estradiol valerate (10 mg/ml) with a dose of 0.5 mg/mouse. On day 2 and 3, mice were inoculated intravaginally with 20 µl of washed suspension of *L. acidophilus* (1×10¹⁰/ml) grown in MRS media. On day 7, second dose of estradiol valerate (0.5 mg/mouse) was injected subcutaneously. On two consecutive days, day 9 and 10, 20 µl of *T. vaginalis* (1×10⁹/ml) suspended in TYI-S-33 medium containing 0.32% Bactoagar were inoculated intravaginally.

Viability of *T. vaginalis* and infection in animals were confirmed daily by preparing wet mounts from the vaginal washes.

Sample collection from infected mice On 5th post infection day, which was, the day of maximum infection (as standardized in earlier experiments of this study) vaginal washes of infected mice were aspirated with the help of autopipette by inoculating 20 µl of PBS (pH 7.2). Samples were taken from all the mice

in a group and were collected in a sterile microtube. The samples were processed for counting *T. vaginalis*, vaginal epithelial cells (VECs) and polymorphonuclear neutrophils (PMNs).

Statistical analysis Data was analyzed for statistical significance using student's T. test and ANOVA.

Results

Table 1 present's data of isolation rate of *T. vaginalis*. The parasite was isolated in 22 (4.4%) women out of 500 clinical samples examined. Out of 272 symptomatic patients in 12 samples, *T. vaginalis* was detected and 10 samples were positive for trichomoniasis out of 228 asymptomatic patients.

Out of total positive samples 4.41% and 4.39% were from symptomatic and asymptomatic patients respectively. Results showed that there was no significant difference between the isolation of *T.vaginalis* from symptomatic and asymptomatic patients. Ten isolates from each group were maintained in axenic form in culture and were used for further experiments in this study.

Table 1: Isolation rate of *T. vaginalis* from symptomatic and asymptomatic patients

Patients	No. of Patients examined (%)	No. of Patients Positive (%)*
Symptomatic	272(54.4)	12(4.41)
Asymptomatic	228(45.6)	10(4.39)
Total	500	22(4.4)

Note: *Isolation percentage S vs. As ($P>0.1$)

Gradual increase up to day 5 followed by decline in all the three parameters, i.e. parasites, PMNs and VECs was observed. On the whole the mice infected with symptomatic patients isolates had significantly high number of all the three parameters in contrast to mice infected with asymptomatic patients' isolates or control animals at all time intervals. The parasite loads in mice infected with symptomatic patients isolates were higher as compared with the mice infected with asymptomatic patients isolates ($P<0.05$) Fifth post infection day (pid) was observed as peak infection one in most experimental animals for both symptomatic and asymptomatic patients isolates. However, parasites were observed up till one month in some of the animals (Fig. 1).

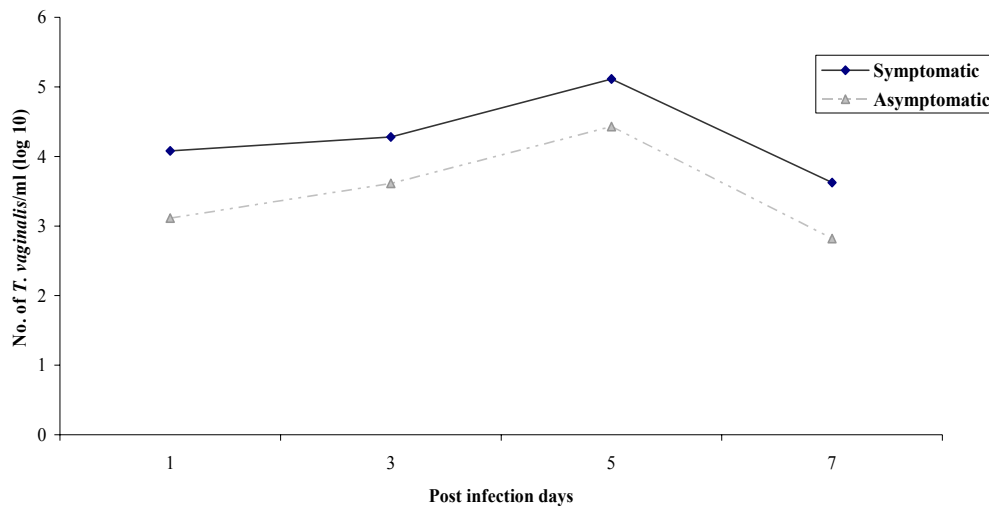


Fig. 1: Comparison of parasite number in BALB/s mice infected with both group of isolates on different post infection days

Mice infected with symptomatic patients isolates indicated significant increase in polymorphs with increase in days of infection as compared with mice infected with asymptomatic patients isolate and control (uninfected) animals (Fig.2). There was significant difference in PMNs count on all pid between mice infected with symptomatic patients isolates

versus control (on 1 pid $P<0.01$ and on 3, 5, 7 pid $P<0.05$), and Asp isolates versus their control ($P<0.05$). However, these differences were not significant when PMNs count from mice infected with symptomatic patients isolates was compared with the counts from mice infected with asymptomatic patients isolates.

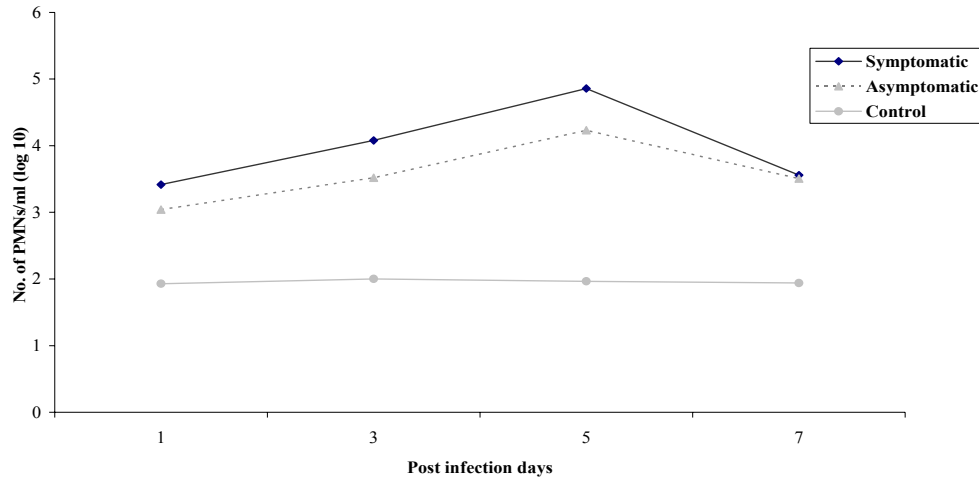


Fig. 2: Comparison of PMNs number in *T. vaginalis* infected mice with both group of isolates on different post infection days

Significant increase in VECs count in mice infected with symptomatic patients isolates as well as asymptomatic patients isolates was observed as compared to their respective controls. Mice infected with symptomatic patients iso-

lates showed significant difference in VECs count on one pid ($p<0.05$) and five pid ($p<0.01$) as compared to asymptomatic patients isolates infected mice. Maximum count was observed on 5th pid in both groups of mice.

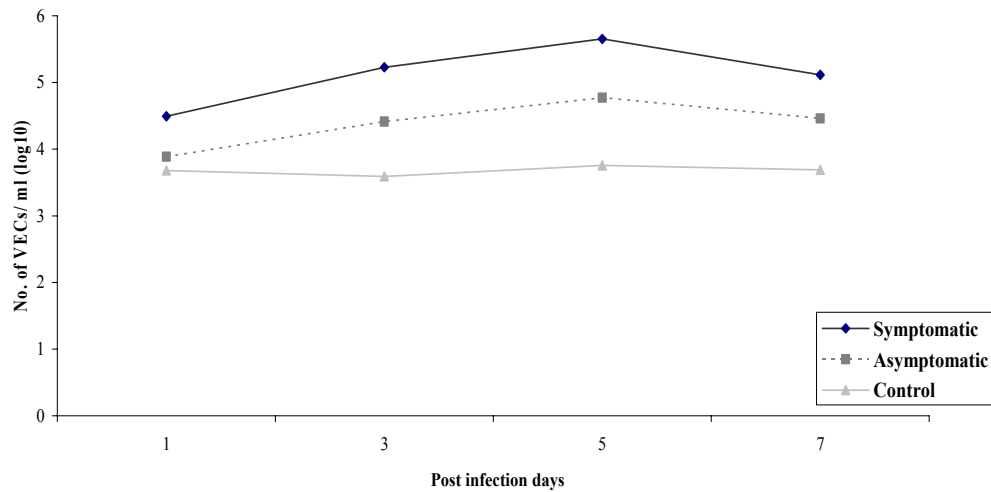


Fig. 3: Comparison of VECs number in *T. vaginalis* infected mice with both group of isolates on different post infection days

Discussion

Trichomoniasis is one of the commonest sexually transmitted disease capable of causing considerable morbidity in infected patients. The infection can occur both in males and females. Most often males may be asymptomatic while females are known to be symptomatic as well as asymptomatic. Microscopic observation of motile protozoa in vaginal secretions or urine has been a traditional and most common diagnostic method for trichomoniasis.

Different strains of *T. vaginalis* have been found to vary with respect to surface carbohydrates (13) and proteins (14).

Rein (15) observed that asymptomatic subjects could become symptomatic within 6 months. This supports the contention that women with asymptomatic trichomoniasis should be treated. This form of the disease is particularly important from the epidemiological point since these individuals are the major source of transmission of the parasite. For establishment of infection, many mechanisms are thought to be involved which include cell to cell adhesion (16); haemolysis (17) excretion of soluble factors like extracellular proteinases (18) and cell detaching factor (19). The host-parasite relationship is very complex and broad range of clinical symptomatology cannot be attributed to a single pathogenic mechanism.

Trichomoniasis is an example of mucosal infection where host parasite interaction plays an important role in deciding the outcome of an infection. To have insight into the pathogenicity of the disease an acceptable model is required. Mouse has been found to be the most popular animal species used in experimental models of *T. vaginalis* infection. Pre-estrogenization has been found to be necessary in order to induce and establish genital infection successfully.

This approach potentiates infection by inducing estrus cycle, which enhances glycogen levels and causes slight drop in vaginal pH. It also decreases cytotoxicity of PMNs and vaginal antibody responses (20). Maintaining of the

infection in the experimental animals required direct demonstration of parasite at frequency time intervals. Peak infection was observed on 5th pid, only in few animals infection continued for 30 days.

Increase in parasite load was reported (21) in mice inoculated with isolates from symptomatic women as compared to those inoculated with isolates from asymptomatic women as shown in this study. Not many reports are available for direct comparison. In addition wet smear prepared from vaginal washes of mice infected with Sp isolates showed significantly more number of VECs than mice infected with Asp isolates. The present study therefore throw attention to the fact that strains isolated from Sp do exhibition increased ability to cause exfoliation of VEC in experimental mice in comparison to that caused by Asp isolates.

The underlined mechanism as to how this happens required to be elucidated. Although antibodies to *T. vaginalis* may be detected in vaginal secretion or serum of the majority of infected women, it may not be detected due to low titers (22). It is not known whether the poor response is due to weak antigenicity of the parasite or to active suppression of immunity. These circulating antibodies have short life and are not able to provide protection against reinfection (23).

The immunological response to invasion of the uro-genital tract by *T. vaginalis* is variable. Recently, it has been stressed that host factors like extensive layer of mucous, nutrient limiting conditions, antibody responses, constant flow of vaginal fluid and normal flora have important role to play in establishment of infection by *T. vaginalis* (24). Recruitment of macrophages and PMNs to the site of infection is the first line of defence as a component of non-specific resistance and immunity (25). Honigberg (26) observed that *T.vaginalis* isolated from Sp were able to stimulate strong chemotactic response towards PMNs as compared to those isolated from Asp.

This resulted in a higher inflammatory response accompanied by leukorrhea in Sp. In an experimental study (21) increase in number of PMNs were observed in pap smear from mice infected with Sp isolates as compared to those infected with Asp isolates and maximum number was observed on 7th pid. In the present study, maximum number of PMNs in vaginal washes of mice was observed on the peak infection day which was 5th pid in mice infected with isolates from symptomatic women although larger number of PMNs were observable in vaginal washes throughout the period of study, i.e. till 7th pid. Demirezen et al. (27) during cytological examination of direct smear from vagina of female patients observed that PMNs increased in patients of trichomoniasis and were found to arrange in a row trailing *T. vaginalis* organism. These leukocytes then pursue the parasite to contact and attempt to engulf them.

Normally, the emigration of leukocytes at the inflammatory site results in killing of parasite by surrounding it. It is accompanied by release of enzyme from both, neutrophils and macrophages. In case of *T. vaginalis*, a ratio of 10 PMNs to 1 *T. vaginalis* in presence of O₂ and complement was shown to kill the parasite by breaking it into pieces and followed by phagocytosis (25).

This may form the basis for future plan of studies in this context to bring out the fact that as to what extent these factors are concerned with the development of symptoms in the patients. Further this will have bearing in developing effective therapy and preventive for human trichomoniasis.

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