

The role of pseudocyst of *Trichomonas vaginalis* in transmission of infection

Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَقَدْ اَعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ
□ وَرَسُولُهُ وَالْمُؤْمِنُونَ

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Abstract

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T.vaginalis, parasitic protists of the urogenital tract, display a trophozoite and a pseudocyst stage. The pseudocyst form appears under unfavorable environmental conditions when the flagella are internalized, and a true cell wall is not formed. Pseudocysts are competent to divide but their role in trichomonas life cycle has not yet confirmed. In this study, the ability of the intra-vaginally inoculated pseudocysts to induce trichomoniasis in infected mice was evaluated in comparison to the trophozoites. Pseudocyst induction was performed physically by thermal-freezing cycle method. The infectivity of pseudocyst was proved by the presence of *T. vaginalis* parasite in mice vaginal washes and by histopathological studies. In *vitro*, *T. vaginalis* trophozoites and pseudocysts were found to possess several different proteinase bands by non-denaturing gelatin-SDS-PAGE (zymography). So, *T. vaginalis* pseudocysts are active forms that can induce trichomoniasis.

Key words: *Trichomonas vaginalis*; pseudocysts; gelatin-SDS-PAGE; proteinase.

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List of Abbreviations

APS	Ammonium persulfate
°C	Degree Celsius.
µg	microgram.
µl	Microliter.
µm	Micrometer.
Bis acrylamide	bis-methylene-acrylamide C7 H10 N2 O2.
CAF	Chromosome aberration factor .
CDF	Cell detaching factor.
Cm	Centimeter.
CP	Cysteine proteinase.
CPLM	Cysteine peptone liver infusion maltose.
D.P.I	days post infection .
DNA	Deoxy ribonucleic acid.
DTT	Dithiothreitol.
EDTA	Ethyl-ene diamine tetra acetic acid.
ELISA	enzyme-linked immune sorbent assay
g	gram.
HIV	Human immuno deficiency virus.
HPV	Human papilloma virus.
Hrs.	Hours.
Ig	Immunoglobulin.
Ig A	Immunoglobulin A.
Ig G	Immunoglobulin G.
IU	International unit.
K₂HPO₄	Dipotassium hydrogen phosphate dibasic.
kDa	Kilodalton.

List of Abbreviations (Cont.)

Kg	Kilogram.
KH₂PO₄	Potassium dihydrogen phosphate monobasic.
L	Liter.
M	Mole.
Ma	Milliampere.
MCA	Modified Columbia Agar.
mg	Milligram.
MGJ	Modified Glycerol jelly.
Min	Minutes.
ml	Milliliter.
Mm	Millimeter.
mM	Millimole.
MW	Molecular weight.
N	Normal.
Na Cl	Sodium chloride.
Na OH	Sodium hydroxide.
Na₂HPO₄	Disodium hydrogen phosphate dibasic.
NaH₂PO₄	Sodium dihydrogen phosphate monobasic.
nm	Nanometer.
No.	Number.
Pap. smear	Papanicolaou smear
PBS	Phosphate buffered saline.
PCR	Polymerase chain reaction.
ppm	Parts per million.
rpm	Round per minute.

List of Abbreviations (Cont.)

SDS	Sodium dodecyl sulphate.
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis.
St.	Standard.
STD	Sexually transmitted disease
<i>T. foetus</i>	<i>Tritrichomonas foetus</i> .
<i>T. vaginalis</i>	<i>Trichomonas vaginalis</i> .
TEMED	Tetra methyl ethyl ene diamine.
TYM	Trypticase, yeast extract, maltose.
V	Voltage.
VECs	Vaginal epithelial cells.

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Protocol of

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Introduction

Trichomonas vaginalis is the most common non-viral sexually transmitted pathogen (**Guenther et al., 2003**). The infection is prevalent in reproductive age women and is associated with vaginitis, endometritis, adnexitis, pyosalpinx, infertility, preterm birth, low birth weight, bacterial vaginosis, and increased risk of cervical cancer, HPV, and HIV infection (**Fichorova, 2009**). In men, its complications include urethritis, prostatitis, epididymitis, and infertility through inflammatory damage or interference with the sperm function (**Bennett et al., 1989**).

In addition to the trophozoite, pseudocyst is another morphological form which is recently identified among genitourinary trichomonads (**Tasca and De carli, 2007**).

Pseudocysts were found in natural culture conditions and also under induction by hydroxyurea or cycles of cooling and warming cultures. They were studied by light microscopy, both scanning and transmission electron microscopy and by using immunofluorescence microscopy (**Benchimol, 2004**). The ultrastructure of the trophozoite was compared to that of the pseudocyst where the latter appears under unfavorable environmental conditions when the flagella are internalized, but a true cell wall is not formed (**Pereira-Neves et al., 2003**).

Mariante et al. (2003) proved that *T. vaginalis* forms pseudocyst under natural and unfavorable conditions. Morphological variability of *T. vaginalis* was determined by different environmental factors such as temperature, pH, oxygen tension, carbohydrate, and contact with other cell types (**Honigberg and Brugeroll, 1990**). The transformation of both the ellipsoid and the spherical (pseudocysts) forms into trophozoite occurred once the parasite was in direct contact with the vaginal epithelial cells (VECs) (**Arroyo et al., 1993; Arroyo and Alderete, 1995**). Besides, the human erythrocytes and microorganisms of the vaginal flora induced morphological changes on *T. vaginalis* (**Rend-on-Maldonado et al., 1998**).

Aim of the work

The aim of the present study is to clarify the role of *T.vaginalis* pseudocyst in transmission of trichomoniasis .

Plan of the work:

1. Collection of vaginal swabs:

- a- Oral consent will be taken from the patients before taking any samples.
- b- Full detailed history including: age, marital status,...ect
- c- Vaginal swabs and/or urine from symptomatic females in the reproductive age groups.
- d- Microscopic examination with direct wet smear method using saline, iodine, eosine and methylene blue.

2. Culture on Trypticase, yeast, and maltose (TYM) media.

3. Assessment of pathogenicity of both trophozoite & pseudocyst:

A. In vitro assessment:

The parasite separated from culture, will be subjected to:

testing the proteinase activity of the cell lysate by gelatin SDS - PAGE (Sodium-dodecyl-sulphate Polyacrylamide gel electrophoresis).

B. In vivo assessment:

Intra-vaginal injection of the parasite in estradiol treated albino mice.

Group I:

Estradiol treated albino mice infected by *T.vaginalis* trophozoite.

Group II:

Estradiol treated albino mice infected by *T.vaginalis* pseudo cyst.

Group III :

(**Control** group) Estradiol treated albino mice without infection.

Then, pathological study of the three groups will be performed and compared.
