

Study of pathogenic role of *Trichomonas tenax* in oral infections

Thesis

Submitted to Faculty of Medicine, Ain Shams University
For Partial Fulfillment of Master Degree in Basic Medical Science
(Parasitology)

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Ain Shams University 2011

دراسة الدور المرضى للمشعرة الفمية في حالات التهابات الفم

رسالة

توطئة للحصول على درجة الماجستير في العلوم الطبية الأساسية (علم الطفيليات)

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Abstract

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Buccal cavity as a compound ecosystem has always been ignored in parasitic infection studies; however it seems that studying oral trichomoniasis is of great importance. The present study aimed to estimate the occurrence of *Trichomonas tenax* in individuals having oral infections in comparison with healthy control and to investigate the possible relationship between pathogenicity of *T.tenax* and its proteolytic activity. The present study was conducted on 100 individuals referred to the faculty of Dentistry, Ain Shams University. They were categorized into 2 groups patients group (G1) and control group (G2). Plaques and/ or calculi samples collected from G1 and saliva collected from G2 were subjected to 1) parasitological examination with direct wet smear; 2) Culture on modified TYI-S-33 medium. Isolates successfully maintained in culture, were used for preparation of *T.tenax* cell lysates, which were furtherly subjected to analysis of protein profile by SDS-PAGE and analysis of proteases by non-denaturing gelatin-SDS-PAGE. The results showed that the total frequency of oral trichomoniasis in G1 was 28.6%, while that of *E.gingivalis* was 8.6%. Modified TYI-S-33 medium was found to be superior to the wet smear method in detecting

Abstract

T. tenax as it was able to detect 100% of the positive samples, while wet smear was able to detect 55% of the positive samples. Comparison of growth kinetics of the seven *T. tenax* isolates showed a wide variability in the growth characteristics. Protein profiles of the seven *T. tenax* isolates revealed a total 53 bands ranged in MW from 5 - 95 kDa using 12% resolution gel. Also, *T. tenax* isolates were found to possess 19 proteinase bands ranged in MW from 14 - 66 kDa by non-denaturing gelatin-SDS-PAGE. The proteolytic bands were intensified by a cysteine proteinase activator as di-thiothreitol (DTT), and totally disappeared by treatment with cysteine proteinase inhibitor (E-64) suggesting that the proteinases were of cysteine proteinases type. It is concluded from the present study that the high frequency of *T. tenax* in periodontal diseases along with the demonstrated proteolytic activity supports the possible pathogenicity of *T. tenax*. Also, the variability in protein profiles and zymography of the *T. tenax* isolates clarify a conclusion that different strains with possibility of variable pathogenic potential may exist.

Key words: oral protozoa; *Trichomonas tenax*; periodontal diseases; SDS-PAGE; gelatin-SDS-PAGE; proteolytic activity; proteinases.

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List of Abbreviation

List of Abbreviations

AIDS	Acquired immune deficiency syndrome.
ATCC	American Type Culture Collection.
AEBSF	4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride.
APS	Ammonium persulfate.
APs	Aspartate proteases.
bisacrylamide	N'N'-bis-methylene-acrylamide C ₇ H ₁₀ N ₂ O ₂ .
<i>B. hominis</i>	<i>Blastocystis hominis</i> .
BSA	Bovine serum albumin.
Cm	Centimeter.
COPD	Chronic obstructive pulmonary diseases.
CFA	Complement fixing antibodies.
<i>C. parvum</i>	<i>Cryptosporidium parvum</i> .
CPLM	Cysteine peptone liver infusion maltose.
CP	Cysteine proteinase.
CPE	Cytopathic effects.
°C	Degree Celsius.
DNA	Deoxyribonucleic acid.
K₂HPO₄	Dipotassium hydrogen phosphate dibasic.
Na₂HPO₄	Disodium hydrogen phosphate dibasic.
DTT	Dithiothreitol.
dsRNA	Double stranded ribonucleic acid.
<i>E.gingivalis</i>	<i>Entamoeba gingivalis</i> .
<i>E.histolytica</i>	<i>Entamoeba histolytica</i> .
EDTA	Ethyl-enediaminetetraacetic acid.
<i>F.hepatica</i>	<i>Fasciola hepatica</i> .

List of Abbreviation

GT	Generation time.
<i>G.lamblia</i>	<i>Giardia lamblia</i> .
Gm	Gram.
Hrs	Hours.
HeLa	Human cervical cancer cells.
HIV	Human immunodeficiency virus.
HEP-2	Human Laryngeal Tumor Cells.
Hcl	Hydrochloric acid.
Ig	Immunoglobulin.
Ig A	Immunoglobulin A.
Ig G	Immunoglobulin G.
Ig M	Immunoglobulin M.
IL-10	Interleukin-10.
IU	International unit.
kDa	Kilodalton.
Kg	Kilogram.
KTB	Kupferberg <i>Trichomonas</i> broth.
LSD	Least significant difference.
<i>L.mexicana</i>	<i>Leishmania mexicana</i> .
L	Liter.
MPs	Metallo-proteases.
µg	microgram.
µl	microliter.
mA	Milliampere.
mg	Milligram.
ml	Milliliter.
Mm	Millimeter.
mM	Millimole.

List of Abbreviation

MGJ	Modified Glycerol jelly.
M	Mole.
MW	Molecular weight.
<i>N. fowleri</i>	<i>Naegleria fowleri</i> .
Nm	Nanometer.
NPV	Negative predictive value.
N	Normal.
No.	Number.
OPB	Oligopeptidase B.
Pap smear	Papanicolaou smear.
Ppm	Parts per million.
PBS	Phosphate buffered saline.
pg	Picogram.
<i>P.falciparum</i>	Plasmodium falciparum.
PCR	Polymerase chain reaction.
PMN	Polymorphnuclear neutrophils.
PVA	Polyvinyl alcohol.
PPV	Positive predictive value.
KH₂PO₄	Potassium dihydrogen phosphate monobasic.
RNA	Ribonucleic acid.
rpm	Round per minute.
<i>S.mansoni</i>	<i>Schistosoma mansoni</i> .
SC	Secretory component.
SIgA	Secretory Immunoglobulin A.
SPs	Serine proteases.
SERA	Serine repeat antigen.
SSURRNA	Small subunit ribosomal RNA.
Na Cl	Sodium chloride.
