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

Thesis

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دراسه الدور المرضى للمشعرة الفمية
في حالات التهابات الفم

رسالة

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

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Abstract

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

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Buccal cavity as a compound ecosystem has always been ignored in parasitic infection studies; however it seems that studying oral trichomonasis 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

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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 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 C7 H10 N2 O2.
B. hominis	*Blastocystis hominis.*
BSA	Bovine serum albumin.
Cm	Centimeter.
COPD	Chronic obstructive pulmonary diseases.
CFA	Complement fixing antibodies.
C. parvum	*Cryptosporidium parvum.*
CPLM	Cysteine peptone liver infusion maltose.
CP	Cysteine proteinase.
CPE	Cytopathic effects.
°C	Degree Celsius.
DNA	Deoxyribonucleic acid.
K_2HPO_4	Dipotassium hydrogen phosphate dibasic.
Na_2HPO_4	Disodium hydrogen phosphate dibasic.
DTT	Dithiothreitol.
dsRNA	Double stranded ribonucleic acid.
E.gingivalis	*Entamoeba gingivalis*.
E.histolytica	*Entamoeba histolytica.*
EDTA	Ethyl-enediaminetetraacetic acid.
F.hepatica	*Fasciola hepatica.*

List of Abbreviation

GT	Generation time.
G.lamblia	*Giardia lamblia*.
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 *Trichomonas* broth.
LSD	Least significant difference.
L.mexicana	*Leishmania mexicana.*
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.
N. fowleri	*Naegleria fowleri.*
Nm	Nanometer.
NPV	Negative predictive value.
N	Normal.
No.	Number.
OPB	Oligopeptidase B.
Pap smear	Papanicoulaou smear.
Ppm	Parts per million.
PBS	Phosphate buffered saline.
pg	Picogram.
P.falciparum	Plasmodium falciparum.
PCR	Polymerase chain reaction.
PMN	Polymorphnuclear neutrophils.
PVA	Polyvinyl alcohol.
PPV	Positive predictive value.
KH_2PO_4	Potassium dihydrogen phosphate monobasic.
RNA	Ribonucleic acid.
rpm	Round per minute.
S.mansoni	*Schistosoma mansoni.*
SC	Secretory component.
SIgA	Secretory Immunoglobulin A.
SPs	Serine proteases.
SERA	Serine repeat antigen.
SSURRNA	Small subunit ribosomal RNA.
Na Cl	Sodium chloride.