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"PHENOTYPIC CHARACTERIZATION OF AMOEBOID FORMS OF *TRICHOMONAS VAGINALIS*"

Field of Study: **MEDICAL PARASITOLOGY**

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ABSTRACT

*Trichomonas vaginalis*is a flagellated protozoan parasite that causes significant health drawbacks to humans, predominantly in women by causing a non-viral sexually transmitted disease known as trichomoniasis. Different morphological properties of *T. vaginalis* were recognized as the key for infections in humans with trophozoites and pseudocysts as the two notable forms. Recently, amoeboid form was identified in suspension cultures and was reported as an integral life cycle of *T. vaginalis*. The present study was conducted to study the phenotypic characteristics of amoeboid forms in suspension culture i.e. growth profile, evaluating factors that trigger formation of this shape, staining characteristics and transformational changes to amoeboid forms. The study showed that out of nine symptomatic isolates consisting of six cervical neoplasia (CN) and three non-cervical neoplasia (NCN), only the CN isolates showed viable amoeboid forms. Amoeboid forms were distinguished based on morphological properties such as formation of pseudopodia-like cytoplasmic extensions, non-motile, none or inactive flagella, flattened, larger in size with abnormal shapes and presence of more inclusion-like bodies. Growth profile of amoeboid forms showed an initial formation in the culture on day one itself with significantly optimal count on day three cultures with total mean average of 3.22 $\pm$ 0.11 x 10^4 *T. vaginalis*/ml ($p < 0.05$) for all CN isolates (CN1 – CN6) and none in NCN isolates (NCN2 – NCN4). This was mainly attributed to the virulence and high proliferation rate of *T. vaginalis* in CN isolates compared to NCN isolates. The study also confirmed a significant formation of amoeboid forms in suspension cultures of CN isolates (CN1 – CN6) under the three growth parameters tested. Firstly, optimum amoeboid forms were observed on day 3 when the growth media were overcrowded with parasites with mean average of 9.88 $\pm$ 0.50 x 10^5 *T. vaginalis*/ml ($p < 0.05$). Increased concentration of horse

serum (15 %) also observed a significant development of amoeboid forms with mean average of 9.95 ± 0.47 x 10^4 *T. vaginalis*/ml ($p < 0.05$). The third parameter on the effect of metronidazole drug (0.0001 mg/ml) reported optimal amoeboid forms with mean average of 3.61 ± 0.06 x 10^4 *T. vaginalis*/ml ($p < 0.05$). The study for the first time introduced Modified Field's stains as a rapid detection method (15 seconds) compared to Giemsa stain. The staining provided better and sharper contrast which differentiated the nucleus and cytoplasm. The staining showed amoeboid forms to have irregular shapes, rough surfaces and with single or multi-nucleated. Using light microscopy, different morphological changes based on size and shapes were observed as the trophozoites of *T. vaginalis* transformed into amoeboid forms. The amoeboid forms measured approximately 25 µm to 27.5 µm in length and 20 µm to 22.5 µm in width and were seen predominantly in cultures after 48 hours culture. The study opened new prospects for further research to understand better the role of amoeboid forms of *T. vaginalis* in contributing to the pathogenesis and development of cervical cancer in women.

ABSTRAK

Trichomonas vaginalis adalah sejenis parasit protozoa berflagela yang menyebabkan kelemahan kesihatan yang ketara kepada manusia, terutamanya di kalangan wanita dengan menyebabkan penyakit bukan-virus kelamin yang dikenali sebagai trikomoniasis. Pelbagai jenis morfologi yang dimiliki oleh *T. vaginalis* diiktiraf sebagai kunci untuk menghasilkan jangkitan kepada manusia dengan trofozoit dan pseudosista sebagai dua bentuk yang ketara. Baru-baru ini, satu lagi morfologi baru *T. vaginalis*, iaitu bentuk amoeboid telah dikenal pasti dalam kultur dan telah dilaporkan sebagai kitaran hayat penting *T. vaginalis* yang boleh dikaitkan dengan unsur patogenik. Kajian ini telah dijalankan untuk menjustifikasikan hasil dapatan ini dengan mengkaji ciri-ciri fenotip bentuk amoeboid dalam kultur berdasarkan profil pertumbuhan, menganalisis faktor-faktor yang mencetuskan kewujudan bentuk ini, kaedah pewarnaan serta memerhatikan perubahan morfologi yang berlaku dalam usaha untuk mencapai bentuk amoeboid ini. Kajian ini menunjukkan bahawa daripada sembilan pesakit bersimptom terdiri daripada enam isolat neoplasia serviks (CN) dan tiga lagi isolat bukan neoplasia serviks (NCN), hanya isolat CN mempunyai ciri bentuk amoeboid yang viabel. Bentuk amoeboid dikenal pasti berdasarkan lanjutan pseudopodia sitoplasma, nipis, tidak mobil atau tiada atau flagela yang tidak aktif, saiz yang besar dengan bentuk tidak sekata dan banyak granul kecil. Profil pertumbuhan bentuk amoeboid menunjukkan kewujudan bentuk ini dalam kultur seawal pada hari kedua dan kiraan menjadi lebih optima dan signifikan pada hari ketiga kultur dengan jumlah purata $3.22 \pm 0.11 \times 10^4$ *T. vaginalis*/ml ($p < 0.05$) bagi semua isolat CN (CN1 – CN6) tetapi tiada bentuk amoeboid dilihat dalam semua kultur isolat NCN (NCN2 – NCN4). Ini disebabkan oleh sifat virulen dan kadar reproduksi tinggi untuk menambahkan sel dalam isolat CN berbanding dengan isolat NCN. Penyelidikan ini juga mengesahkan pembentukan

morfologi amoeboid yang signifikan dalam kultur isolat CN apabila persekitaran pertumbuhan diletakkan di bawah tiga jenis tekanan. Pertama, lebihan sel parasite (10^5 sel/ml) membentuk bentuk amoeboid dengan optimum pada hari ketiga dengan jumlah purata sebanyak $9.88 \pm 0.50 \times 10^5$ *T. vaginalis*/ml ($p < 0.05$). Peningkatan konsentrasi serum kuda (15 %) juga menunjukkan pembentukan signifikan bentuk amoeboid dengan jumlah purata sebanyak $9.95 \pm 0.47 \times 10^4$ *T. vaginalis*/ml ($p < 0.05$). Ketiga, kesan dadah metronidazole (0.0001 mg/ml) juga merekodkan jumlah pembentukan amoeboid yang tinggi dengan jumlah purata sebanyak $3.61 \pm 0.06 \times 10^4$ *T. vaginalis*/ml ($p < 0.05$). Kajian ini memperkenalkan buat kali pertama pewarna Field yang diubahsuai ("Modified Field Stain") sebagai pewarna pantas (15 saat) berbanding pewarna Giemsa untuk mentakrifkan fenotip bentuk amoeboid dengan memberikan kontras yang lebih tajam untuk membezakan nucleus dan sitoplasma. Pewarna Field juga dapat mengesahkan bentuk amoeboid sebagai tidak teratur, permukaan kasar, satu nukleus atau multinukleus dan saiz yang lebih besar. Menggunakan mikroskop cahaya, perubahan morfologi yang nyata diperhatikan dari aspek saiz dan bentuk apabila trofozoit *T. vaginalis* berubah kepada bentuk amoeboid. Bentuk amoeboid berukur dengan anggaran panjang dalam lingkungan 25 µm hingga 27.5 µm manakala lebar dalam 20µm hingga 22.5 µm dan jelas diperhatikan selepas 48 jam dalam kultur yang tertekan. Kajian ini membuka prospek baru tentang morfologi *T. vaginalis* yang berbeza ini dan boleh menyumbang ke arah pathogenesis dan perkembangan kanser serviks di kalangan wanita.

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LIST OF SYMBOLS AND ABBREVIATIONS

AP	Adhesins proteins
CDC	Centers for Disease and Control
CDF	Cell detaching factor
CN	Cervical neoplasia/Cervical intraepithelial neoplasia
CP	Cysteine proteinases
^{o}C	Degree of Celcius
dsRNA	Double-stranded Ribonucleic Acid
DPX	DinButyl phthalate and Xylene
ELISA	Enzyme-linked Immunosorbent Assay
FDA	Food and Drug Administrations
Fig.	Figure
g	gram
%	Percent
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
HVEC	Human vaginal epithelial cell
Ig	Immunoglobulin
KCl	Potassium chloride
kDa	Kilodalton
KH_2PO^4	Potassium dihydrogen phosphate
µl	Microlitre
µm	Micrometer

ml	Mililitre
mg	Miligram
NCN	Non-cervical neoplasia/ Non-cervical intraepithelial neoplasia
n.d.	no date
PAP	Papanicolaou
PCR	Polymerase Chain Reaction
pH	Power of Hydrogen
rpm	revolutions per minute
S.D	Standard deviation
SPSS	Statistical Package for the Social Sciences
STD	Sexually transmitted disease
STI	Sexually transmitted infection
WHO	World Health Organization

LIST OF APPENDICES