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Trichomonas vaginalis, Genetic Variation, and Pathogenicity: a Systematic Review

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***Abstract*- Trichomoniasis is a sexually transmitted infection that has been associated with acquired immunodeficiency syndrome and cervical cancer. The objective of the present study was to conduct a systematic review of the literature to identify the genetic variations of *Trichomonas vaginalis* and their correlations with the vaginal environment in the human host. Two electronic databases, PubMed and the Virtual Health Library (*Biblioteca Virtual de Saúde*), were searched using Medical Subject Headings (MeSH) and Health Sciences Descriptors (DeCS), respectively. The terms "*T. vaginalis* and *genetic variation*" and "*T. vaginalis* and *oxidative stress*" were searched to identify relevant original articles. The inclusion and exclusion criteria established took into consideration the specific characteristics of each article, thus guaranteeing the quality of the papers selected (the first and second tests of relevance). Two principal types of population structure of *T. vaginalis* were reported in the papers: type 1 and type 2. Type 1 parasites were associated with pathogenicity, as shown by findings of increased viral loads in human immunodeficiency virus (HIV)-positive women.**

***Index Terms*— Genetic variation, immunosuppression, oxidative stress, *Trichomonas vaginalis*.**

I. INTRODUCTION

Trichomoniasis, a sexually transmitted infection that is principally associated with the acquired immunodeficiency syndrome (AIDS) and with cervical cancer, has been increasing in prevalence in countries of Africa and Latin America [1-4]. The protozoan *Trichomonas vaginalis* inhabits the human vagina and is morphologically characterized by four flagella and one undulating membrane. The movement of the flagella characterizes the active forms, while immobility denotes a less active stage that, nevertheless, conserves the parasite's mitotic capacity [5].

With a genome of approximately 160 megabases and a capacity for expansion, *T. vaginalis* possesses a wide, diverse set of repeated, mobile genetic elements that provide it with a mechanism for expansion and diversity [6, 7]. Furthermore, as an anaerobe, *T. vaginalis* can respond to oxidative stress, reducing the oxygen resulting from the Krebs cycle through its reductive enzymes [8, 9].

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The objective of the present study was to conduct a systematic review to identify the principal genetic types of *T. vaginalis*. Correlating the parasite with oxidative stress may explain the putative association between trichomoniasis and immunodeficiency status.

II. METHODS

A systematic review of the literature was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) specifications [10]. Papers identified in indexed database were selected to answer the following question: "Is there a genetic variation in *Trichomonas vaginalis* infection?".

The database PubMed and Virtual Health Library (*Biblioteca Virtual de Saúde*) were electronically searched for Medical Subject Headings (MeSH) and Health Science Descriptors (DeCS), respectively. The term initially used was "*Trichomonas vaginalis* and *genetic variation*". The papers identified were included in the review only if the inclusion and exclusion criteria were met and the papers were found to be relevant to the study objective (Table 1). The first test included the best papers found with the first cross-referencing. In the second test, articles were selected using a second pair of keywords: "*Trichomonas vaginalis* and *oxidative stress"*.

All the studies included in the selection were published between 2006 and 2016 except for 2 papers from 2004 and 2005, respectively. These latter articles were considered relevant to the present study and since the search yielded so few papers they were included.

III. RESULTS

A. The first test of relevance

The search for articles in the English language using the term "*Trichomonas vaginalis* and *genetic variation*" retrieved 103 papers. Of these, 70 were identified using MeSH and 33 with DeCS (Table 2).

Of the 70 articles retrieved from PubMed using the first MeSH term "*Trichomonas vaginalis* and *genetic variation*", 19 were included (Table 2-a) and 51 excluded. Of the 51 papers excluded, 36 were published prior to 2006, 8 dealt with unrelated aspects, 3 were short communications, 2 were

Table 1: Form for the application of the relevant tests.

Form for the application of the test 1	Yes	No
Inclusion Criteria: 1) "Is there a genetic variation in *Trichomonas vaginalis* infection?"		
Exclusion Criteria: 1) Do not obey inclusion criteria 2) Editorial, letter or review? Reason for the exclusion:		
Form for the application of the test 2		
Inclusion Criteria: 1) "Is there a correlation between *Trichomonas vaginalis* and oxidative stress?"		
Exclusion Criteria: 1) Do not obey inclusion criteria 2) Insuficient data for evaluation? Reason for the exclusion:		

Table 2: Retrieved articles per database.

Data Base	Date	Time	Total
MeSH	12/07/2016	8:00	70
	12/07/2016	9:30	06
DeSC	12/07/2016	10:30	33
	12/07/2016	11:30	16
Total			125

review articles, 1 was a case study, and 1 was in another language.

Of the 33 articles retrieved from the Virtual Health Library using the DeCS "*Trichomonas vaginalis* and *genetic variation",* 1 was included (Table 2-a), while the remaining 32 were excluded. Of the papers excluded, 16 were published prior to 2006, 9 dealt with unrelated aspects, 6 were duplicates and 1 was a short communication. Of those initially excluded because they were published before 2006, an exception was made for one paper published in 2004, which was considered relevant.

B. *The second test of relevance*

The second MeSH term "*Trichomonas vaginalis* and *oxidative stress"* retrieved six papers from PubMed, with four of these being included (Table 2-b) and two excluded. The two that were excluded were review articles. Of the four papers included, one was published in 2005 but nevertheless was considered relevant to the study.

The second DeSC "*Trichomonas vaginalis* and *oxidative stress"* retrieved 16 papers from the Virtual Health Library, one of which was included (Table 2-b), while the remaining 15 were excluded. Of these, 10 dealt with unrelated aspects, 4 were duplicates, and 1 consisted of a review article.

IV. DISCUSSION

Two principal types of population structure of *T. vaginalis* were reported in the selected articles: type 1 and type 2. Type 1 corresponds to the wild type G3 strain, which is the type most commonly found at wet mount microscopy. This corresponds to the most active stage, since positivity at wet mount microscopy is associated with movement of the flagella of the parasite. In addition, Matini et al. found the CT66 mutation in internal transcribed spacer (ITS) region 1. This consists of the substitution of cytosine by thymine at nucleotide location 66 [13, 15, 16].

Figure 1: Study flowchart: Relevance test 1

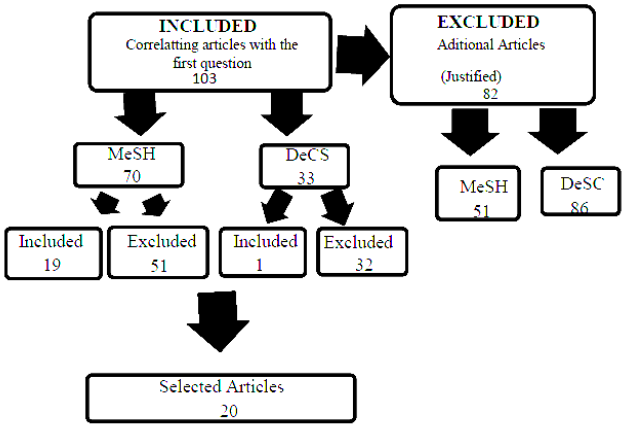
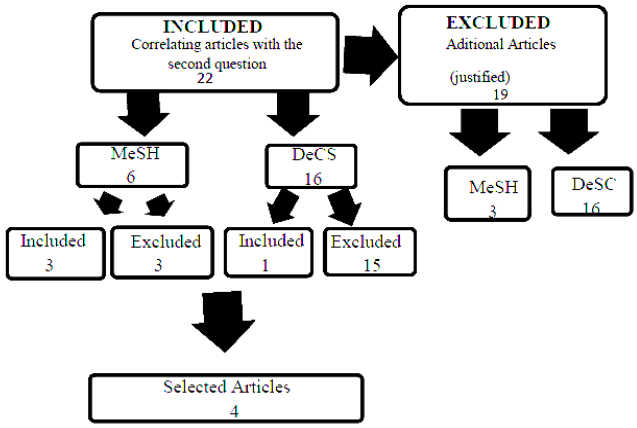


Figure 2: Study flowchart: Relevance test 2



A statistically significant ($p=0.01$) and clinically important association was found between type 1 *T. vaginalis* infections and increased viral loads in HIV-positive women in use of antiretroviral therapy (ART). Nevertheless, compared to population-based groups (HIV-positive women and controls), there was no genetic difference between the species [15].

Type 2 *T. vaginalis* has been found to be more resistant to metronidazole. Resistance was related to the inhibition of nitroreductase (ntr_{tv}). Variations in the DNA sequences affected only one base in the *ntr4*, *ntr6*, *ntr9*, and *ntr10* haplotypes of *T. vaginalis*. The *ntr4* and *ntr5* haplotypes were associated with each other. The minimum lethal doses of metronidazole were significantly associated with the *ntr5* genotype [13]. On the other hand, Type 1 parasites were more susceptible to infection by the *T. vaginalis* virus (TVV) and were more susceptible to metronidazole compared to the type 2 parasites, confirming the greater resistance of this second type [17].

The virulence of *T. vaginalis* may be impacted by the presence of the TVV because of the ability of the virus to trigger variations in phenotype. The study conducted by Fraga et al. included isolates with TVV in Group 2. The presence of TVV was associated with the genetic polymorphism. Of the 37 clinical isolates of *T. vaginalis* analyzed, 21 were infected with the genetic group 1 [20].

The active state of *T. vaginalis* may reflect its greater capacity for phagocytosis. Bearing in mind that a phagocyte

Table 2a: Qualitative analysis of the articles selected using the Medical Subject Headings (MeSH): *"Trichomonas vaginalis & genetic variation"*.

Authors	Country of Publication/ Year	Diagnostic Methods	Journal	Impact factor
Momemi et al.[11]	Germany/2015	Culture, PCR[1]-RFLP[2]	Exp. Parasitol.	1.64
Whole et al.[12]	United Kingdom/2014	Culture, RT-PCR[3]	BCM Genom.	3.68
Paulish-Miller et al.[13]	United States/2014	Culture, SNP[4]	Ant.microb.Ag. Chem.	4.47
Lara-González et al.[14]	United States/2014	IMAC[5], IFI[6]	Prot. Struct. Bioinf.	2.63
Conrad et al.[15]	United Kingdom/2013	Culture, PCR[1], MS[7]	Sex. Transm. Infect.	3.40
Matini et al.[16]	Malasia/2012	Culture, SSCP-PCR[8]	Trop. Biomed.	0.85
Horváthova et al.[6]	United Kigdom/2012	Culture, cDNA Microarray[9] with EST[10]	Genom. Biol. Evol.	4.22
Figueirôa-Ângulo et al.[8]	United Kingdom/2012	Culture, RT-PCR, Antibodies assay[11]	Parasitol.	2.56
Cornelius et al.[17]	United States/2012	Culture, PCR, MLST[12]	J. Clin.Microb.	3.99
Kay et al.[7]	United States/2012	Culture, RT-PCR, confocal microscopy	PLoS Negl. Trop. Dis.	4.44
Fraga et al.[18]	Germany/2012	Culture, RAPD[13], PCR multiplex	Exp. Parasitol.	1.64
Conrad et al.[19]	United States/2012	Culture, PCR, MS	PLoS Negl. Trop. Dis.	4.44
Fraga et al.[20]	Germany/2011	Culture, PCR, RAPD	Exp.Parasitol.	1.64
Conrad et al.[21]	United States/2011	Culture, MS, SCG[14], FISH[15]	Molec. Biochem. Parasitol.	2.06
Cui et al.[22]	United States/2010	Culture, PCR, RT-PCR	PLoS Negl. Trop. Dis.	4.44
Rivera et al.[23]	Germany/2009	Culture, PCR	Parasitol. Res.	2.03
Meade et al.[24]	United States/2009	Culture, PCR, RFLP	Am. J. Trop. Med. Hyg.	2.69
Crucitti et al.[25]	Switzerland/2008	Culture, nested PCR-RFLP	Clin. Microbiol. Infect.	4.57
Brown et al.[26]	United States/2007	Culture, PCR, SDS-PAGE[16]	Mol. Microb.	3.76
Bradis et al.[29]	United Kingdom/2012	Culture, PCR, RT-PCR	Mob.DNA	2.52

1. PCR: Polimerase Chain Reaction. 2. RFLP: Restriction Fragment Lenghth Polymorphism. 3. RT-PCR: Reverse Transcription Polimerase Chain Reaction. 4. SNP: Single-Nucleotide Polymorphism. 5. IMAC: Immobilized Metal Affinity Chromatography. 6. IFI: Immunofluorescence Induect. 7. MS:: Microssatellite molecular markers. 8. SSP-PCR: Single Specific Primer-Polimerase Chain Reaction. 9. cDNA: complementary desoxiribonucleonde acid. 10. EST: Express Sequence Tags. 11. Antibodies assay with specific antibodies. 12. MLST- Multilocus Sequence Typing. 13. RAPD: Random Amplified Polymorphic DNA. 14. SCG: Single-copy genes. 15. FISH: Fluorescence In situ Hybridization. 16. SDS-PAGE: Polyacrylamide gel electrophoresis.

may contain a series of different microorganisms including viruses, bacteria and fungi, *T. vaginalis* may act as a carrier, spreading and facilitating other infections in the host organism [19, 20].

T. vaginalis may be associated with the endosymbiotic presence of the Mollicutes class of bacteria. Species of Mollicutes were detected in 7.5% of isolates (3/40). *Mycoplasma hominis* was detected in the C12 and A163 isolates. Isolate C147 was positive for Mollicutes class but negative for the other four species tested. However, it was the only one that reflected clinical symptomatology [18].

The endosymbiosis of TVV increases the virulence of the parasite in the host organism, since there are immunogenic proteins on the surface of *T. vaginalis* that may flourish due to the presence of the endosymbiont, as suggested by Conrad et al. [19] Fichorova et al. argued that placental insufficiency in pregnant women may be the result of the *T. vaginalis* virus inducing the production of interleukin 8 [33].

Different manifestations of trichomoniasis may be due to the variations in the host's individual factors. The molecular behavior of *T. vaginalis* has already been evaluated based on clinical signs and symptoms. Rivera et al. conducted a study with a population of asymptomatic sex workers in different regions of the Philippines. Sequencing of the 5.8SrDNA gene and the internal transcriber spacer (ITS) regions was conducted using 57 isolates of *T. vaginalis*. Using the G3 strain as reference, similarity was 99-100% for the isolates from the Philippines. Of the 57 isolates, 46 belonged to H1 and this had the best representation, with 6 types of sequences. Seven isolates belonged to H4, while of the four other types each one represented a single isolate. The sequences of types 2 and 6 (H2 and H6) differed from the reference strain by only one nucleotide position. Types 1 and 4 did not reflect random mutations alone, but, rather, *T. vaginalis* strains possibly associated with the patients' asymptomatic state [23].

Woehle et al. experimentally validated the expression of a set of long, non-coding RNAs (lncRNAs) in the T1 strain, in

Table 2b: Qualitative analysis of the articles selected using the Health Sciences Descriptors (DeCS): *"Trichomonas vaginalis & oxidative stress"*

Authors	Country of Publication/Year	Diagnostic Methods	Journal	Impact factor
Smutná et al.[28]	China/2014	Culture, Western blot, Molecular cloning, TLC[17], Ferrous oxidation in xilenol-orange assay, MRI[18]	Ant. Agents Chem.	2.72
Gould et al.[29]	Japan/2013	Culture, PCR	Int. Parasitol.	1.86
Pütz et al.[30]	United States/2005	Culture, MCF[19], biochemical assays, Eletrophoresis 2D, Isoeletric Focusing, Mass spectrometry, DIF[20]	J. Biochem. Parasitol.	2.06
Coombs et al.[31]	United States/2004	Cultura, Clonagem molecular, PCR	J. Biol. Chem.	4.57

17- TLC: Thin-Layer Chromatography. 18- MRI: Magnetic Ressonance Imaging. 19- MCF: Method of Cellular Fractionation. 20- DFI: Direct Immunofluorescence

the virulent T016 strain and in the highly virulent FMV1 strain. Six cases of lncRNAs were found in each of the three strains tested. One set of pseudogenes included 7% of all the transcripts analyzed [12].

T. vaginalis infection was classified into three different types according with its intensity: mild, moderate or severe. The asymptomatic patients had four specific markers. The 1724 pb marker was found in all the isolates from women in whom the infection was mild, while 2418 was found in isolates from women in whom infection was moderate and two genetic markers (534 and 1360 pb) were amplified only in the group of women with severe infections [32].

Genes that encode many protein families in the species genome may explain its adaptation to the different microenvironments of the host, given the multiplication of the parasite by cloning (mitosis) and the expansion of its genome [27]. *T. vaginalis* contains 2 *tpi* genes that encode triose phosphate isomerase (TIM). This enzyme catalyzes the isomerization between glyceraldehyde-3-phosphate and dihydroxyacetone phosphate in the parasite. At the end of the study, results showed that the tpi2 transcript was approximately 3.3 times more abundant than tpi1 [8].

Lara-González et al. reported the TvTIM1 enzyme to be a more stable protein than TvTIM2, since the former contains an isoleucine whereas the latter contains a valine. The removal of a methyl group causes a decrease in the stability of various isoleucine (I1e) mutations in valines, since the difference in stability is associated with the loss of van der Waals interactions and with the difference in hydrophobia [14].

The ATP-binding cassette (ABC) transporter gene family expands considerably in *T. vaginalis*, including expansion of membrane transport and flow. According with the topology of each protein and based on the number and order of transmembrane domains and of the nucleotide binding domain (NDB), *T. vaginalis* was found not to encode full-length transporters. Furthermore, a large number of orphan ABC genes were found in *T. vaginalis*, denoting the suppression of stop codons by *T. vaginalis*. The study concluded that the absence of full-length ABC transporters in *T. vaginalis* is unique among the sequenced eukaryotes [7].

Six genes were selected for a phylogenetic analysis. Different evolutionary relationships were found between strains: with GP63a, PMS1, M1h 1a, CRN, GP63b and LLF4 representing potentially virulent genes and a potential drug target for later analysis. The set of data on the surface protein consisted of 4,182 positions aligned with 30 local polymorphisms translated to 24 varying locations in the protein sequence, with one having three variant amino acids.

The set of data consisted of 5,619 unequivocally aligned positions including 34 polymorphic positions, translating to 23 varying locations in the protein sequence. The phylogeny of the maintenance gene sustains the B7RC2 and F1623 strains [21].

Actin plays a role in the pathogenicity of *T. vaginalis*. It is presumed that its ability to change morphologically is related to its virulence, and the importance of cytoskeletal integrity must be emphasized [11]. Momeni et al. amplified the actin gene using nested polymerase chain reaction (PCR) and showed an 1100 pb fragment in 7 isolates of sequenced *T. vaginalis*. According with agarose gel electrophoresis examination, no change occurred in the length of 45 amplicons. Four different types of *T. vaginalis* (G, E, He and I) were identified in the 45 isolates. The most common type was G (51.1%) [11].

Oxidative stress in the human body may play a role in the state of activation of *T. vaginalis*. Since these parasites are anaerobic, they can act by reducing oxygen and other metabolites through a reductive system linked to the enzymes thioredoxin, peroxiredoxin and rubrerythrin [8, 30, 31]. The malate enzyme (ME) and pyruvate-ferredoxin oxidoreductase (PFOR) catalyze the oxidative decarboxylation of malate and pyruvate in the hydrogenosomes of *T. vaginalis* and may be able to act in the absence of the iron element, since the expression of 4 ME and 2 PFOR genes has been demonstrated under these circumstances [6]. Smutná et al. characterized the catalytic properties of an iron-sulfur flavoprotein (TvIsf) present in *T. vaginalis* hydrogenosomes, and found that it had a short extension of 8 amino acids, with an amino-terminal (N-terminal) that is not present in homologous prokaryotes. The TvIsf flavoprotein, acting within the hydrogenosomes and consequently causing activation, may represent a defense mechanism against xenobiotics in the host organism [28].

V. CONCLUSION

Two principal types of population structure of *T. vaginalis* were found: type 1 and type 2. *T. vaginalis* can reduce the oxygen resulting from cellular respiration to remain in anaerobiosis. The conditions of oxidative stress may thus lead to possible activation of the parasite in the vaginal environment. The genetic variations in the parasite correspond to the genetic polymorphisms that originate from the wild type G3 strain (type 1), which are the result of resistance to metronidazole, of horizontal transferences caused by endosymbionts and/or of the simple adaptive process due to an environmental condition caused by adequate immunity (type 2).

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