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SEXUALLY TRANSMITTED CUTANEOUS AMOEBIASIS: AN EMERGING PARASITIC DISEASE?

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Keywords:	E. histolytica, E. dispar, Cutaneous amoebiasis, Genetic variants, Genotype

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Abstract

Cutaneous amoebiasis is the least common clinical form of human amoebiasis. Cases usually correspond to patients with deep skin lesions in areas such as; anal, perinea, vulvae, vaginal and genitalia, as well as the diaper area in babies with intestinal diarrhea due to *E. histolytica* infection. In adults the most common location of cutaneous amoebiasis is the abdominal area as a consequence of the abdominal wall spontaneous involvement of amoebic liver abscess or as a complication of an amoebic liver abscess drainage procedure. In Mexico this pathology before the late 1980's was occasionally observed. However, in the last decades, most of the documented cases of this condition are among sexually transmitted amoebiasis cases. This case of penis cutaneous amoebiasis is the first documented case where the presence of both *E. histolytica* and *E. dispar* species was detected; this finding indicates that mixed infections are more than an isolate observation in human invasive amoebiasis.

Introduction

Cutaneous amoebiasis was first described in 1892 by Nasse in a patient with an amoebic liver abscess that was complicated after drainage causing extended areas of ulceration and necrosis of abdominal skin, subcutaneous tissue and muscles. No evidence of the presence of trophozoites is mentioned in the original article, but the Nasses´s clinical diagnosis was amoebic liver abscess and cutaneous amoebiasis (*1*). This condition is the least common clinical form of human amoebiasis and in general the consequence of Entamoeba infection of previously damaged skin barrier. Ngai and Frazier reviewed the available literature back to 1936 finding 27 reported cases from China, Indo-china and United States (*2*); thereafter the number of reported cases of cutaneous amoebiasis decreases substantially to 11 cases in1941 (*3-9*). Magaña et al. (2008) recorded 26 documented cases of cutaneous amoebiasis from the clinical files of the pediatric dermatology department in the General Hospital of Mexico from the Health Ministry and from the pathology department of the Medical Specialties Hospital of the Mexican Institute of Social Security (IMSS) at the XXI Century Medical Center (*10*) However, the majority of the reported cases occurred from 1969 to the late 80's. Cases correspond to patients with deep skin lesions in areas such as; anal, perinea, vulvae, vaginal and genitalia, as well as the diaper area in babies with intestinal diarrhea due to *E. histolytica* infection. In adults the most common location of cutaneous amoebiasis is the abdominal area as a consequence of the abdominal wall spontaneous involvement of amoebic liver abscess or as a complication of an amoebic liver abscess drainage procedure (*11-15*). Another type of infection is the sexually transmitted form of amoebiasis. In 1978 this type of amoebiasis was well documented by Hurwitz and Owen in San Francisco, the authors mention that during 1976 the reported incidence of laboratory confirmed cases of amoebiasis was 101 as compared to 8 cases in 1970 (*16*). Thereafter, the documented cases of this clinical form of amoebiasis decreased substantially; cutaneous amoebiasis was almost unknown to recent

generations of physicians in Mexico. Even though, cutaneous amoebiasis is a rare disease, most of the documented cases of this condition reported in the international literature in the last decades have been sexually transmitted amoebiasis cases (*17-21*). In the last 12 months two cases of sexually transmitted perianal amoebiasis were documented by Medina-Murillo and Rodríguez-Wong (2011) in Mexico (22).

Our group has been interested in the study of the molecular epidemiology of human amoebiasis (*24-27*), the characterization of *E. histolytica* and *E. dispar* genetic variants most prevalent in Mexico and their relation to the clinical outcome of the infection. The interest in the present case of a sexually transmitted penis cutaneous amoebiasis is to stress the importance of this clinical form of amoebic disease in the differential diagnosis of both genitalia and perinea ulcerative lesion in men and females. A timely diagnosis and specific treatment are essential to avoid serious anatomical damages and ensure the healing lesions without future functional sequels.

Clinical case

The patient is a 38 year old male, born in the state of Veracruz, living in Mexico City for the last 3 years, secondary school, chronic smoking and alcoholism. The disease started in the first weeks of December 2010, with prepuce and penis edema, areas of indurations, constant pain, urethral blood discharge and blood clot in urine. The physician in charge indicated antibiotic therapy (levofloxaxine 500mg every 24 hr for ten days, ceftriaxone 1g bid for five days and clindamicyn 300mg for ten days) Three days after, one of several prepuces' indurations areas spontaneously drained bloody purulent material withoutodor, the patient referred no fever or general discomfort. The patient was admitted in the urology department of Mexico´s General Hospital SSa. The physician in charge asked for a clinical evaluation from the dermatology department. The clinical exploration showed localized dermatosis

affecting the trunk genital region involving the penis at its shaft and glands distal portion, the scrotum and the pubis. Dermatosis was characterized by a painful and progressively growing ulcer with erythematous bottom and irregular borders of necrotic appearance covered by fobrinoid discharge. Deformation of the penis was evident giving the impression of a circumcised penis. The patient was submitted to surgical debridement and for biopsy of the lesion. The routine clinical laboratory tests were under normal limits. The patient was discharged after 7 days antibiotic treatment with cefuroxime (750 mg IV every 8 h); the biopsy reported balanitis with acute and chronic inflammatory cells infiltration and multiple pyogenic micro abscesses and the absence of neoplasic cells.

The patient returned to the hospital with the previous lesion enlarged, all the prepuce was absent, the posterior side of penis showed a large area of skin erosion with serous and purulent material(Figure 1a). There was an open abscess in the supra pubic area draining a non-fetid bloody and purulent material, the scrota was also affected with multiple ulcers (Figure 1b). At this point the physician in charge consulted with us for a possible *E. histolytica* infection.

The relevant laboratory findings were; high levels of anti-amoebic IgG antibody detected by ELISA technique previously described (*28*)D.O. 0.65 cut of values 0.525), microscopic detection of *E. histolytica/ E. dispar* in biopsy specimen, PCR characterization of Entamoeba species in the bloody purulent material indicating a mixed *E. histolytica* and *E. dispar* cutaneous infection acquired by sexual transmission. The patient was treated with metronidazole (500 mg IV every 8 hr) and ceftriaxone (1 g IV every 12 hr). After 30 days of anti-amoebic and antibiotic treatment, the abscesses healed and the presence of large areas of granulation tissue were observed, however, the loss of skin was severe involving all the

penis, the patient is now followed to undergo reconstructive surgery and skin autologous transplant.

Material and methods

Microscopic detection of Entamoeba trophozoites

A second biopsy of an ulcer border located in the penis was obtained and fixed in para-phormaldheyde during 24 h, tissue was segmented and then de-hydrated and embedded in paraffin, the slides (4-5 µM) were cut with a microtome (Leica RM 2145) the slides were stained with Hematoxilin-eosin (HE) and Peryodic acid stain (PAS)techniques (29). The slides were observed at 10X and 40 X magnifications in a light microscope.

Genotyping and Phylogenetic reconstructions of genetic markers of Entamoeba species

DNA was extracted from the purulent and bloody secretion specimens taken from different sites of the genital region by tissue scrapping using the "DNA Easy Tissue" Kit (Qiagen, Valencia, CA, USA), following the manufacturer's instructions. Two tRNA gene-linked short tandem repeats were amplified in DNA using primers species-specific for *E. histolytica* and *E. dispar* as previously described (*30-31*). The primers used to amplify molecular markers for *E. dispar* were StgaD3-D5 and NKD3-D5 and for *E. histolytica* the correspondent StgaH3-H5 and NKH3-H5. The reaction and PCR conditions for amplification of these molecular targets were previously described (*31*). The sequencing reactions had a total volume of 15 µl consisting of 2 µl of the Big Dye Terminator Sequencing kit (Applied Biosystems), 1.6 µM of primer and 5 µl of the purified amplified product. The amplification conditions were: 1 cycle of 5 min at 95 °C, 45 cycles of 10 sec at 95 °C, 10 sec at 50 °C and 4 min at 60 °C. Sequencing was performed in a capillary sequencer (ABI-Avant 100). Sequences were manually verified with the BioEdit program (*32*). Taxonomic identity was established by

comparing the obtained sequences against the GenBank (NCBI) data. Sequences were aligned using the Clustal X software program (*33*). Phylogenetic reconstruction for both molecular markers, Stga and NK was carried out through the neighbor-joining method using the MEGA program, version 3.0. (*34*). The substitution model for each one of the markers was Kimura 2-P.

Results and Discussion

The presence of Entamoeba trophozoites were observed in the second biopsy taken from the largest ulcer border, the biopsy was performed after two days of metronidazole therapy, fortunately the presence of trophozoites was clearly identified in the slides stained with the PAS technique (Figure 2) showing the characteristic dark red color. We may observe the absence of dermis and epidermis tissues and the large infiltration of inflammatory cells in particular mononuclear and polymorphic nuclear leukocytes. The histolytic capacity of *E. histolytica* has been well documented since the first reports of cutaneous amoebiasis (*10-13*). The present case is representative of the evolution of lesions without specific anti-amoebic treatment and the irreversible character of damage. Even though clinical diagnosis of this form of invasive amoebiasis is relatively simple (microscopic detection of trophozoites in tissue specimens and high titles of serum anti-amoebic antibodies), it should betaken into account for the differential diagnosis of ulcerative lesions of human genitalia, in particular in endemic areas of amoebiasis and in high risk groups. From an epidemiological point of view the extensive study of such cases allows us to trace transmission, sources of infection and characterize prevalent genotypes of *E. histolytica* and *E. dispar* variants in endemic countries. This case highlights the complexity of the Entamoeba human infection. Previously our group reported cases of amoebic liver abscess where the presence of both *E. histolytica* and *E.*

dispar infection was demonstrated by the PCR technique using different molecular markers (*35*).Coincidentally the amoebic lesions in this patient were also co-infected by pyogenic bacteria *Enterobacter cloacae* and *Staphylococcus aureus*, this was the case of pyogenic and mixed liver abscess(amoebic-pyogenic) reported previously(*35*), where we propose that the invasive behavior of Entamoeba species may be related with the consortium of Entamoebas and bacteria. This case of cutaneous amoebiasis is the first case where the presence of both species o Entamoeba has been demonstrated (Figure 3a). The genotyping of *E. histolytica* variants reported was performed using as molecular targets the intergenic tRNA regions StgaH3-H5 (Figure S4) and NKH3-H5 (Gene Bank accession number JN191599) andStgaD3-D5 (Figure S4) and NKD3-D5 (Gene Bank accession number JN191598) for *E. dispar;* the sequence analysis and the phylogenetic reconstruction are shown in Figure 3b, each species was found in a different clade in both molecular markers. Also, the polymorphism analysis demonstrated that both species has distinct genotypes related to the type strains and other previously reported sequences. This case is without any doubt a simultaneous invasive infection due to both Entamoeba species. These findings support that mixed infections are more than an isolate observation in human amoebiasis.

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Biographical Sketch

Patricia Moran is MD and a PhD fellow in Biological Sciences of the Metropolitan University in Mexico City campus Xochimilco.

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Figure legends

Figure 1

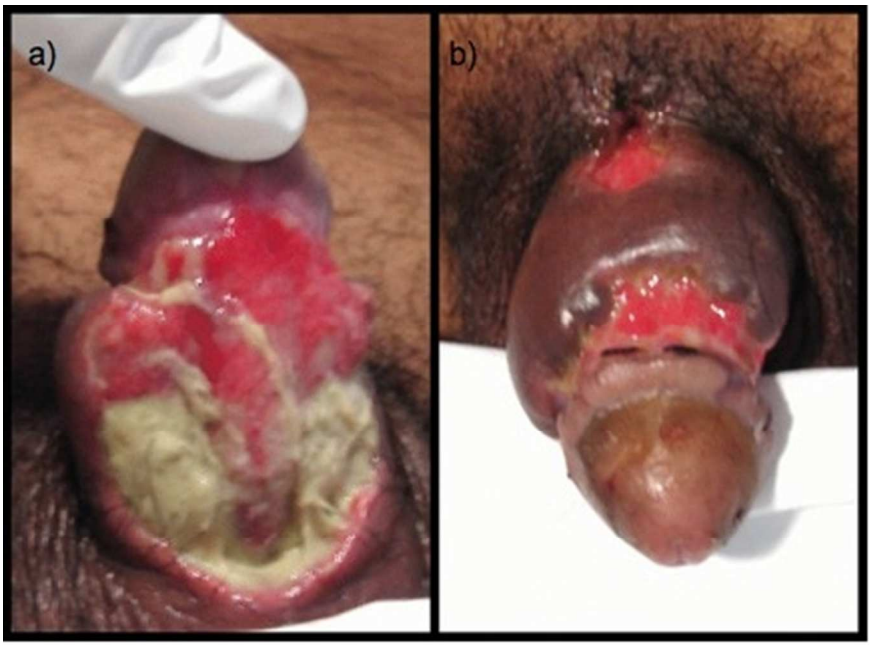
Macroscopic lesions. a), View of dorsal region of penis showing a large ulcerative lesion with irregular borders. Part of the ulcer is covered by fibrinoid discharge. b) Anterior view of, genital region with the ulcerative lesion in pubis, ulcerative lesion in the penis with necrotic lesions in the borders and the total absence of prepuce.

Figure 2

Microphotography of biopsy specimen obtained after 10 days of metronidazole treatment. The tissue slides were stained with the periodic acid technique (PAS). -The presence of an important infiltration of mononuclear inflammatory cells and red stained trophozoites of *E. histolytica /E. dispar,* was observed, some of them seriously damaged as a consequence of 10 days of metronidazole treatment.

Figure 3

Phylogenetic reconstruction through the neighbor-joining method from the intergenic tRNA NKH3-H5 region (Gene Bank accession number JN191599) for *E. histolytica* and NKD3-D5 region (Gene Bank accession number JN191598) for *E. dispar.* Bootstraps values with 2500 replications are indicated close of the node numbers. Bar (0.02) shows nucleotide substitutions at each position. Each species was found in a different clade in both molecular markers.StgaH3-H5 andStgaD3-D5 sequences are included as a supplement material in Figure S4.



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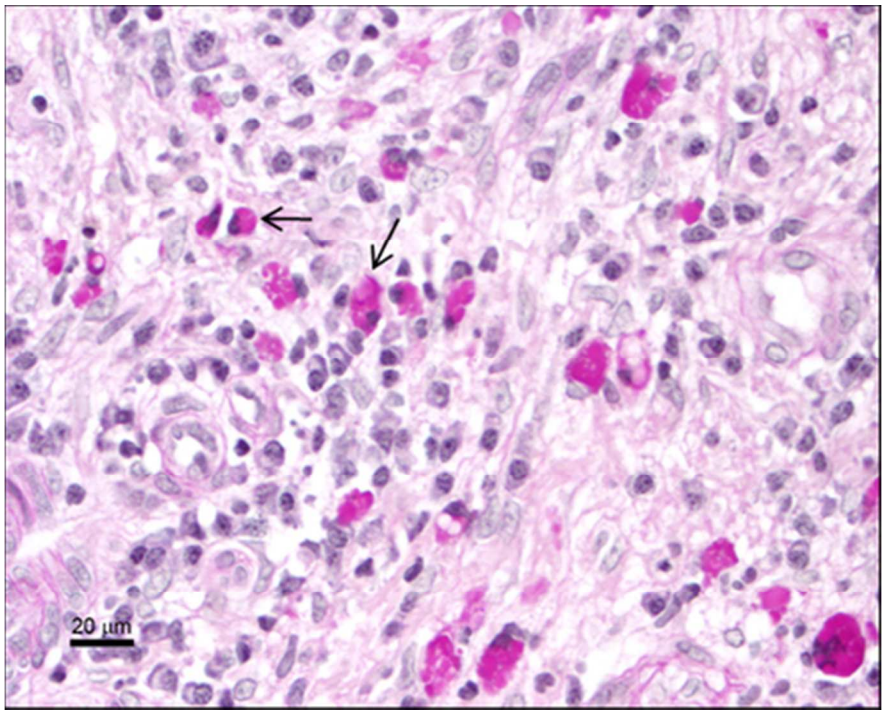

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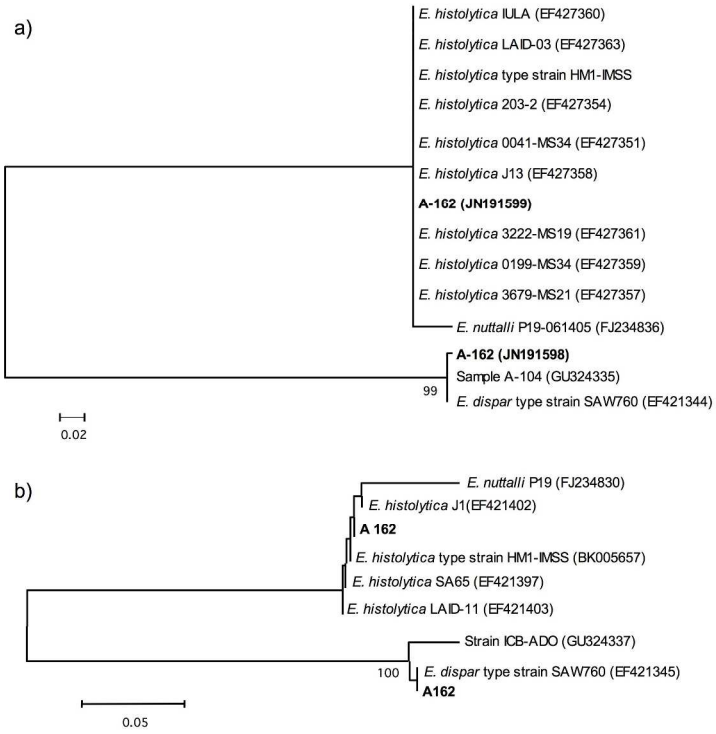

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Figure 4s

>A162StgaD (*Entamoeba dispar*)
AATCCTGCCACTGTCGTACTCTTTTTATAAAGAATATAAATAATATAGTAAA
AAAGAAATATAATAAATAAAAATAAATATATATAAGAAAGGAATAATATAT
ATTTTATTGTAACAAAAAATAGTATAATAAATAGTCATATATATACTTATAA
AAAGAAATATTATAGTATAAAAAGTGAACTCTTCAACGGGGAT
>A162StgaH (*Entamoeba histolytica*)
AATCCTGCCACTGTCGTAATCTTTTTATAAACATAGAAATAATAGAAATAAT
AGAAATAATAGAAATAATAGAAATAATAGAAAATATAGAAATAATAAAAA
GTTATAATATAGTAAGAAAAAATAGTATAATATATAAACATATAAACATAT
AAGGATATAAACATATAAAAGTATAAAAAGAGAACTCTTCAACGGGGATTA
A