Differences in Histoplasmosis in Patients with Acquired Immunodeficiency Syndrome in the United States and Brazil

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Demographic and clinical parameters among patients with acquired immunodeficiency syndrome and histoplasmosis in Brazil and United States were compared. The Brazilian isolates were typed by restriction-fragment length polymorphism analysis and were DNA fingerprinted by random amplification of polymorphic DNA (RAPD)-polymerase chain reaction (PCR). Skin lesions occurred in 66% of Brazilian case patients, compared with 1%–7% of US case patients. Of 21 treated case patients, 4 (19%) died, a rate similar to that of the US case patients (5%–13%). By nuclear gene typing, the Brazilian isolates were equally divided between South American classes 5 and 6, and RAPD-PCR showed 18 distinct genetic fingerprints in 20 isolates. Skin lesions are more common in infection with class 5 or 6 organisms than with class 2 Histoplasma capsulatum. The role of genetic differences in the organism as a cause for the clinical differences requires investigation.

Histoplasmosis is the most common of the endemic mycoses in patients with AIDS in the United States [1]. Histoplasmosis also is common in certain parts of Latin America, posing a significant problem to patients with AIDS [2]. Skin lesions, uncommon in US cases (<10%) [1, 3], occur in 38%–85% of cases reported from Latin America [4–7]. Although these differences may reflect reporting bias, delayed diagnosis, or differences in host immune response among different ethnic groups, they also could result from genetic differences changing the pathobiology of the organism.

Histoplasma capsulatum can be classified into 6 classes by restriction-fragment length polymorphism (RFLP) analysis using the nuclear geneyps-3: (1) the Downs strain, (2) the standard North American variety, (3) the first Panamanian variety, (4) a single variety from Florida soil, (5) a Central and South America variety, and (6) a second Panamanian variety [8]. It is possible that genetic differences among strains of H. capsulatum may alter the pathogenesis and clinical manifestations of histoplasmosis. In this study, we compare clinical features of patients from the United States with those from patients in Brazil and present some genetic analysis of the Brazilian isolates.

Materials and Methods

Patients

US multicenter cohort. A case patient was defined by having a culture positive for H. capsulatum, a urine- and/or serum–positive H. capsulatum polysaccharide antigen test, or histopathologic findings revealing organisms consistent with H. capsulatum [9].

Indianapolis cohort. One hundred and fifty-five case patients with disseminated histoplasmosis were identified by review of culture and Histoplasma antigen results from Indianapolis during the 1988–1995 outbreak [10].

Liposomal amphotericin B treatment cohort. Seventy-three patients were enrolled between August 1995 and April 1999 in a multicenter trial that compared liposomal amphotericin B with the deoxycholate formulation of amphotericin B for treatment of histoplasmosis [11].
**Table 1.** Comparison of demographic characteristics and CD4 cell count in patients with histoplasmosis in the United States vs. Brazil.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prospective multicenter observational study [9] (n = 92)</th>
<th>Retrospective Indianapolis severity study [10] (n = 155)</th>
<th>Prospective liposomal amphotericin study [11] (n = 75)</th>
<th>Brazilian cohort (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>54 (60)</td>
<td>147 (95)</td>
<td>64 (88)</td>
<td>26 (90)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>50 (54)</td>
<td>37 (24)</td>
<td>38 (52)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>10 (10)</td>
<td>2 (1)</td>
<td>11 (15)</td>
<td>0</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>22 (19–49)</td>
<td>32 (23–62)</td>
<td>31 (16–68)</td>
<td>28 (19–45)</td>
</tr>
<tr>
<td>Median CD4 cell count, cells/mL (range)</td>
<td>25 (0–490)</td>
<td>32 (2–638)</td>
<td>18 (1–182)</td>
<td>&lt;50 (4–134)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%), unless otherwise indicated. The denominator varied for different parameters and can be determined by dividing the number in the brackets by the percentage listed for that observation.

**South American cohort.** Twenty-nine patients were enrolled between October 1993 and May 1996 into a study to characterize the clinical features of histoplasmosis in patients with AIDS. All HIV-infected case patients with cultures growing *H. capsulatum* at the mycology laboratories of the Instituto de Infectologia Emilio Ribas and the Reference Center for AIDS/STD in São Paulo were eligible for the study.

**Laboratory Methods**

*Southern blot using theyps-3 nuclear gene.* Genomic DNA was extracted and purified by use of a technique described elsewhere [12]. Genomic DNA (2 μg) was digested at 37°C with *Bgl*II (Promega Biotech), according to the manufacturer’s recommended conditions. Digests were electrophoresed in Tris-borate buffer [13] and were transferred to a nylon-supported membrane (Nytran; Schleicher & Schuell) by use of the Southern blotting method [14]. The filter was hybridized overnight with 106 cpm nick-translated 1.85 Kb *Hind*III yps-3 probe fragment/mL of hybridization buffer containing 0.5 M sodium phosphate (pH 7.2), 7% SDS, 1% bovine serum albumin, and 1 mM EDTA at 65°C. The blot was washed initially in 2X standard saline citrate (SSC)/0.1% SDS to remove excess probe. The blot was next washed twice in 0.1% SSC/0.1% SDS at 65°C before autoradiography [8].

*Polymerase chain reaction (PCR) method.* Primers used were H1-GGCCTAGAGTCTTGCAAGCACAACGTGC, H2-ACGTTCATGATAACTTCTGGCTCTCATC, and H3-AAGCTTGCATTGTGTTTCCGTATACTGT [15]. The final volume of each PCR mixture was 20 μL. Each PCR mix included 1 U of AmpliTaq DNA polymerase, 0.250 mM each dNTP, PCR buffer with 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, and 0.0001 U gelatin per volume, an additional 5.0 mM MgCl2, primer H1 or H2 at 150 nM or H3 at 100 nM, and 50 ng *H. capsulatum* DNA. Reactions occurred in MicroAmp 0.5-mL eppendorf tubes (Perkin Elmer). Amplification was carried out in a Perkin-Elmer Cetus GeneAmp PCR System 2400.

Low-stringency cycling was conducted for 4 cycles at 94°C for 5 min, 40°C for 5 min, and 72°C for 4 min. High-stringency cycling was conducted for 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min.

The PCR products were run in a 1.3% agarose gel, total volume of 125 mL containing 8 μL of ethidium bromide at 125 V for 20 min followed by 150 V for 2 h in Tris-Borate-EDTA buffer. The isolates were considered different only if the banding patterns were reproducibly distinguishable.

**Statistical Methods**

Continuous baseline characteristics and outcomes were compared by use of the Kruskal-Wallis test for ordered measurements, and the χ2 test was used to compare categorical characteristics. A significance level of α = .05 (2-sided) was used to test all hypotheses.

**Results**

*Comparison of baseline characteristics.* Baseline characteristics are summarized in table 1. Male sex predominated in all 4 groups. Black race was a factor in 24%–54% of the case patients in the United States; however, race was more difficult to assess in the Brazilian cohort. Most Brazilians are of mixed European lineage, with a strong Portuguese background and thus are not Hispanic. Dark-skinned persons in Brazil, termed “pardo,” usually have mixed African and European ancestry. There were 2 dark-skinned persons in the Brazilian cohort. The median age was similar in all 3 cohorts, as was the CD4 cell count.

*Comparison of clinical findings, cultures, and serology.* Skin lesions were present in the majority of the Brazil case patients but uncommon in the US case patients (table 2). Skin lesions were localized in 6 (21%) and widespread in 13 (45%) case patients and were most commonly described as papular and can be determined by dividing the number in the brackets by the percentage listed for that observation.

**Table 2.** Comparison of clinical findings in patients with histoplasmosis in the United States vs. Brazil.

<table>
<thead>
<tr>
<th>Clinical finding</th>
<th>Prospective multicenter observational study [9] (n = 92)</th>
<th>Retrospective Indianapolis severity study [10] (n = 155)</th>
<th>Prospective liposomal amphotericin study [11] (n = 75)</th>
<th>Brazilian cohort (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin involvement</td>
<td>4 (4)</td>
<td>2 (1)</td>
<td>5 (7)</td>
<td>19 (66)a</td>
</tr>
<tr>
<td>Gastrointestinal involvement</td>
<td>7 (8)</td>
<td>3 (2)</td>
<td>6 (8)</td>
<td>7 (24)a</td>
</tr>
<tr>
<td>Bone/joint involvement</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Brain/meningeal involvement</td>
<td>1 (1)</td>
<td>6 (4)</td>
<td>2 (3)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Pulmonary involvement on X-ray</td>
<td>49 (43)</td>
<td>73 (47)</td>
<td>31 (42)</td>
<td>17 (71)b</td>
</tr>
<tr>
<td>Baseline culture positive</td>
<td>36 (51)</td>
<td>105 (81)</td>
<td>54 (78)</td>
<td>29 (100)</td>
</tr>
<tr>
<td>Seropositive</td>
<td>NA</td>
<td>46 (67)</td>
<td>NA</td>
<td>7 (33)c</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%). The denominator varied for different parameter and can be determined by dividing the number in the brackets by the percent listed for that observation. P values represents a comparison of the combined US cohort with the Brazilian cohort. NA, not applicable.

a P < .0001.

b P = .11.

c P = .02.
cases is shown in figure 1. Gastrointestinal findings also were more common in the Brazilian case patients (24%) than in the US case patients (2%–8%; \( P = .003 \)). Although endoscopy was not performed to confirm the diagnosis, histoplasmosis was assumed to be the cause, because the gastrointestinal complaints subsided in response to amphotericin B therapy. Pulmonary involvement also was more common in the Brazilian cohort and included diffuse infiltrates in 63% and focal infiltrates in 8% of the case patients.

Culture positivity ranged from 51% to 81% in the US cohorts, compared with 100% in the Brazilian cohort (positive culture was a requirement for inclusion into the Brazilian cohort). Seropositivity for anti- \( H. capsulatum \) antibodies, measured by immunodiffusion and complement fixation, was lower in the Brazilian case patients (33%) than in the US patients (67%; \( P = .02 \)).

**Comparison of treatment and outcome.** The proportion of patients hospitalized was greater in the combined US groups than in the Brazilian cohort (\( P = .0003 \); table 3). The mortality rate was higher in the Brazilian cohort (39%) than in the combined US groups (10%; \( P < .0001 \)), but 8 of the Brazilian case patients died before therapy was administered.

Among the patients treated with amphotericin B, the mortality was 25% in the Brazilian cohort (4/16), compared with 23% in the US cohort (5/22). Itraconazole was commonly used, either as induction or maintenance therapy in all 4 groups, whereas fluconazole and ketoconazole were prescribed less frequently.

<table>
<thead>
<tr>
<th>Treatment and outcome</th>
<th>Prospective multicenter observational study [9] (n = 92)</th>
<th>Retrospective Indianapolis severity study [10] (n = 155)</th>
<th>Prospective liposomal amphotericin study [11] (n = 73)</th>
<th>Brazilian cohort (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment with</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>59 (64)</td>
<td>47 (30)</td>
<td>73 (100)</td>
<td>16 (57)</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>84 (91)</td>
<td>34 (22)</td>
<td>57 (78)</td>
<td>15 (56)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>19 (21)</td>
<td>41 (26)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>3 (3)</td>
<td>18 (12)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Result</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalized</td>
<td>80 (87)</td>
<td>143 (92)</td>
<td>73 (100)</td>
<td>21 (75)^ab</td>
</tr>
<tr>
<td>Death</td>
<td>11 (12)</td>
<td>20 (13)</td>
<td>4 (5)</td>
<td>11 (39)^ac</td>
</tr>
</tbody>
</table>

**NOTE.** \( P \) value represents a comparison of the combined US cohorts with the Brazilian cohort.

Of the 22 treated cases, 4 died (19%).

Among the patients treated with amphotericin B, the mortality was 25% in the Brazilian cohort (4/16), compared with 23% in the US cohort (5/22). Itraconazole was commonly used, either as induction or maintenance therapy in all 4 groups, whereas fluconazole and ketoconazole were prescribed less frequently.

yps-3 *Nuclear gene classification and DNA fingerprint analysis.* RFLP analysis of the yps-3 gene showed 10 of the Brazilian isolates to belong to class 5 and 10 to class 6. The polymorphic \( BgII \) restriction fragments of 12 and 2.41 kb were observed in the class 5 isolates, whereas a single \( BgII \) fragment containing yps-3 resulted in a 4.2-kb band in all class 6 isolates (figure 2).

When random amplification of polymorphic DNA (RAPD–PCR [16] was used, 10 of the class 5 isolates resolved into 9 distinct amplified patterns (figure 3). Seven isolates were genetically distinct when all 3 primers were used, and 2 pairs that were similar with the H1 primer, H201 and H197, proved unique when analyzed with the 2 other primers. By means of this RAPD analysis, 2 class 5 isolates, H21 and B17, were indistinguishable when all 3 primers were used.

The 10 class 6 isolates were resolved into 9 distinct fingerprints. When the H1 primer was used, M31, H381, and H538 were identical, along with isolates H73 and H134 being indistinguishable, resulting in 7 distinct patterns among the H1 profiles. Patterns formed by both H2 and H3 resulted in 8 distinct fingerprints, but isolates M31, K1312, and H538 were identical with both. M31 and H538 failed to be discriminated with all 3 primers.

**Discussion**

Clinical differences were observed between the Brazilian and US cohorts. Skin involvement was significantly more common in the Brazilian case patients. A variety of skin lesions were observed in the Brazilian case patients, and, in many, the involvement was extensive. The most common skin manifestation was diffuse papuloulcerative lesions with crusting, followed by pustular or nodular lesions. These are similar to the lesions described in patients with histoplasmosis in North America [17–21]. Of note, data from the US case patients were derived...
from retrospective analyses of information collected for other reasons but not to characterize the clinical features of histoplasmosis. As a consequence, atypical lesions may have been overlooked. However, the consistency of the data in the 3 US cohorts along with other reports in the literature suggests that the incidence of these unusual findings is accurately reported. Also, requirement of a positive culture in the Brazilian cohort may have biased that group to include an increased proportion of case patients with skin lesions; the skin lesions would be easier to culture.

Gastrointestinal involvement also appeared to be more common in the Brazilian case patients; however, these findings were based on symptoms, not demonstration of lesions containing *H. capsulatum* organisms by stain or culture. A variety of gastrointestinal manifestations have been described in patients with histoplasmosis [22], and the reticuloendothelial tissue in the gastrointestinal tract is one of the more common sites of dissemination. Whether such manifestations are more common in Brazil is unknown, because diagnostic studies were not done to prove that histoplasmosis was the cause for the gastrointestinal signs and symptoms.

Lung involvement may be more common in the Brazilian case patients. Although the difference did not reach statistical significance, pulmonary involvement was noted in >70% of the Brazilian case patients, compared with less than half in the US case patient. Nodular opacities or diffuse infiltrates were most commonly seen [3]. However, the types of radiographic abnormalities observed in the Brazilian case patients resemble those reported in North American case patients [3, 23].

The mortality rate was higher in the Brazilian cohort, but many of the patients died before treatment could be administered. Death occurred in 5%–13% of the US case patients versus 39% among the Brazilian case patients in the current study. Of importance, 28% of the Brazilian case patients received no antifungal therapy. Of those who were treated, 19% died. Death without therapy suggests that the Brazilian patients presented late during the course of the illness or that histoplasmosis was not suspected and diagnosed in a timely fashion. In an earlier report that described the outcome of amphotericin B treatment, we reported that mortality approached 50% in patients with shock or respiratory failure, compared with only 2% in those with milder illness [1]. These findings support a hypothesis that delayed recognition and treatment until advanced disease portends a poorer outcome, which perhaps accounts for the higher mortality in the Brazilian cohort.

All the Brazilian case patients were infected with South American class 5 or 6 strains of *Histoplasma*, as based on the *yps-3* nuclear gene RFLP typing. Although isolates 1–9 on figure 2 have been grouped into class 5 because of the common 2.41-kb *Bgl*II polymorphism, the last isolate, 10 (H526), provides a distinct *yps-3* RFLP pattern. This isolate lacks the larger 12-kb fragment found in other members of the group, and a new polymorphic fragment has been generated. Restriction enzyme–generated fragment polymorphisms are caused by typically small point mutations that create or disrupt the sequence-specific recognition site, although insertions also have been reported [24]. Although it is possible that isolate H526 represents a new and previously undescribed *Histoplasma* class, the pattern is clearly distinct from that found in the class 6 group and is most consistent with generation of a new *Bgl*II restriction site by point mutation within the larger 12-kb *Bgl*II fragment. Despite a lack of common exposure, 2 pairs of the 20 Brazilian isolates displayed identical fingerprinting by RAPD-PCR. Of interest, none of the patients with identical fingerprints were related or presented at the same time, making exposure to a common source unlikely. However, when Kasuga et al. [25] compared 46 isolates from throughout the world, North American class 2 showed far less variation than South American isolates, which was confirmed in their ongoing evaluation of >137 isolates. Phylogenetic clades (species) of *H. capsulatum* from North America appear to have passed through bottlenecks associated with glaciation from which phylogenetic species in lowland tropics (South America and Africa) escaped, which explains the greater diversity of the South American clades (T. Kasuga and J. W. Taylor, personal communication).

Recent work by Taylor et al. [26] suggests that assignment of 3 varieties of *Histoplasma* is phylogenetically meaningless but, rather, that at least 8 separate species exist. Our findings suggest unique pathogenic characteristics among the common North American and the South American species of *H. capsulatum*.
Figure 3. Random amplification of polymorphic DNA–polymerase chain reaction DNA fingerprints of the Brazilian strains of *Histoplasma capsulatum* from patients. *A*, H1 primer; *B*, H2 primer; and *C*, H3 primer.

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References

5. Rocha MM, Severo LC. Disseminated histoplasmosis in patients with ac-