

Vulval Involvement in Acquired Immunodeficiency Syndrome-Associated Disseminated Histoplasmosis

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Abstract

Background: Female genital tract, including vulval, histoplasmosis is reported rarely despite an increased propensity for cutaneous involvement by disseminated histoplasmosis (DH), even in patients with acquired immunodeficiency syndrome (AIDS).

Methods: Sixteen year retrospective study investigating vulval involvement by histoplasmosis.

Results: Of 239 patients with DH, 6 had vulval involvement and were confirmed to have HIV infection and AIDS. Seventeen biopsies (9 vulval, 8 extravulval) from these 6 patients form the study cohort. Patients 1 to 4 had simultaneous vulval (5) and extravulval (5) cutaneous biopsies. Eight cutaneous biopsies demonstrated diffuse dermal infiltration by histiocytes containing budding yeasts of *H. capsulatum* variant *capsulatum* (HCVC). A single thigh lesion demonstrated diffuse dermal karyorrhexis and myriad extracellular HCVC and a lymph node were diffusely effaced by histiocytes containing HCVC. Patient 5 had concomitant, co-lesional disseminated Kaposi sarcoma and HCVC infection. Patient 6 had 2 initial biopsies that demonstrated *H. capsulatum* variant *duboisii* (HCVD). Three biopsies of persistent facial and vulval plaques and a vulval ulcer, despite amphotericin treatment, confirmed HCVD, Cytomegalovirus and HCVD and Herpes simplex virus infection in each of the persistent lesions, respectively. Patients 2, 3 and 4 died before treatment was commenced. Patient 5 was lost to follow-up and did not receive any treatment. Patient 1 had resolution of DH following treatment with itraconazole. Persistent cutaneous lesions (Patient 6) healed with aciclovir and ganciclovir but uterine cervical squamous carcinoma was diagnosed 6 months later.

Conclusion: Vulval involvement by histoplasmosis shares overlapping clinical features with many infections and tumors. Vulval biopsies are pivotal for diagnosis and allied therapeutic monitoring, particularly in the context of AIDS-associated co-morbid pathology.

Keywords: Vulva; Histoplasmosis; HIV; AIDS; *H. capsulatum*

Introduction

Human histoplasmosis is caused by 2 subspecies of *Histoplasma capsulatum*, *H. capsulatum* variant *capsulatum* (HCVC) that causes classical histoplasmosis and *H. capsulatum* variant *duboisii* (HCVD) that causes African histoplasmosis [1]. The former is a deep mycosis that exists worldwide but is endemic to regions in the Western Hemisphere including the central United States, Latin American and Caribbean countries [2-4]. Whilst 90-95% of new infections are asymptomatic, the rest present with symptomatic acute pulmonary, disseminated and chronic pulmonary forms of the disease [5]. Disseminated histoplasmosis (DH), an acquired immunodeficiency syndrome (AIDS)-defining disease [6] that mainly involves the liver, spleen, lymph nodes and bone marrow [7], was documented rarely in the pre-AIDS era. The mucocutaneous manifestations of DH are reported in 10-25% of patients in the USA and in up to 80% of patients in Latin America [8]. In contrast to the relatively more common reports of genitourinary histoplasmosis in men [9], histoplasmosis of the female genital tract is reported rarely (Table 1) [9-15], even in epidemic regions for histoplasmosis or acquired immunodeficiency syndrome (AIDS). Increased propensity for cutaneous involvement in DH is also reported [2,7,8], but to date, vulval involvement by HCVC in patients with AIDS has been documented only once in the English-language literature [13]. Furthermore, although cutaneous involvement is a characteristic of African histoplasmosis [16], cutaneous vulval infection by HCVD is unreported to date.

In reporting 6 patients with disseminated, including vulval, histoplasmosis that served as the sentinel of HIV infection and AIDS, we describe the histomorphological spectrum of vulval histoplasmosis (VH), and emphasize clinically unrecognized histoplasmosis as a cause of the vulval lesions. Additionally, we document the hitherto unrecorded impact of vulval co-infections as a cause of a suboptimal response to antifungal therapy, vulval co-lesional occurrence of histoplasmosis and Kaposi sarcoma, and cutaneous vulval involvement by HCVD.

Materials and Methods

The database of the Department of Anatomical Pathology, University of KwaZulu-Natal and the National Health Laboratory Service, Durban South Africa was accessed to identify all vulval biopsies that were

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Received September 24, 2016; **Accepted** November 03, 2016; **Published** November 10, 2016

Citation: Ramdial PK, Sing Y, Ramburan A, Nargan K, Singh B, et al. (2016) Vulval Involvement in Acquired Immunodeficiency Syndrome-Associated Disseminated Histoplasmosis. J AIDS Clin Res 7: 632. doi: [10.4172/2155-6113.1000632](https://doi.org/10.4172/2155-6113.1000632)

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Year	Age	Presentation	Genital Histoplasmosis		E-GH	S/illness	Treatment	Outcome
			Site	CM				
1959 [10]	28	Postpartum fever	LM	ulcers	Liver, GI, lungs, spleen, kidney		IV nystatin	Died
1969 [11]	86	PVB	Vagina	Ulcer	joints	nil	AmphotericinB	Resolved
1973 [14]	27	Vulvar ulcers	LMi+LM:R	Ulcers	ND	I-LN	No treatment	Resolved
1997 [15]	63	Dysuria, PVB	Vagina, LMi:R+L, clitoris	Ulcers	ND	DM	Ketoconazole	Resolved
2000 [12]	32	Irregular menses	Ovary (L)	Mass	ND	SLE, C-CF	Ketoconazole	Resolved
2010 [9]	42	CS, rash, PVB	Cervix	Necrotic lesions	BM/skin	AIDS	LAB/itraconazole	ND
2013 [13]	50	LM pain, dysuria	LM (L), vagina	Ulcers	ND	AIDS, I-LN	LAB, HAART	NCH

Key: Age: Patient Age in Years; AIDS: Acquired Immunodeficiency Syndrome; BM: Bone Marrow; C-CF: Colocecal Fistula; CM: Clinical Morphology; CS: Non-specific Constitutional Symptoms (fatigue, fever, abdominal pain); DM: Diabetes Mellitus; E-GH: Extra-genital Histoplasmosis; GI: Gastro-Intestinal Tract; HAART: Highly Active Anti-Retroviral Therapy; I-LN: Inguinal Lymphadenopathy; IV: Intravenous; L: Left; LAB: Liposomal Amphotericin B; LMi: Labium Minus; LM: Labium Majus; NCH: Near Complete Healing; PVB: Per Vaginal Bleeding; ND: Not Documented; R: Right; SLE: Systemic Lupus Erythematosus; S/illness: Systemic Illnesses; Year: Year of Publication

Table 1: Literature review of histoplasmosis of the female genital tract [9-15].

No.	A/CD4	SI	Extra-vulval lesions			Vulval lesions		Diagnosis	Histo	Outcome
			CM/(Biopsy Site)	D/DD	Histo	CM(Size):Site				
1	35/16	PTB	D-*P/N/U (Arm)	TB	DHI	S-*PI (2 cm):*R LM:	TB/Cancer	DHI	Re	
2	30/55	Past PTB	D-*P/N (Leg) *Inguinal LN	TB/NHL	DHI/DHI	M-*U (1.5 cm):*L LM M-U(0.5cm): R+L LMi:	GI	DHI	DWOT	
3	45/34	ND	S-*N (Thigh)	Cancer/TB	DK	M-*PI (2 cm)*U (2 cm):*R LM	Syphilis/TB	DHI	DWOT	
4	22/3	ND	D-*P/N/U (Neck)	TB	DHI/DK	M-*P/PI/N:R+*L LM	Cancer/TB	DHI	DWOT	
5	26/46	ND	D-*P*/N/U (back)	NHL	KS/DHI	M-*P/N (haemorrhagic):*R LM	KS/NHL	KS/DHI	LTFU	
6	38/42	Cervix SCC	D-*P/PI/N (Neck)	TB	DHI	M-*U (5 mm):RLM+*LLM	TB/GI	DHI	Re/LTFU	
			S-*PI (Face)	TB/Ca	DHI	M-*U (5 mm)+*PI (3 cm):*R LM	Cancer/KS	DHI		

Key: A: Age, CD4:CD4 T-Lymphocytes in mm³, CM: Clinical Morphology; D: Disseminated; D/DD: Clinical Diagnosis/Differential Diagnosis; DHI: Diffuse Histiocytic in Filtrate with Intracytoplasmic *H. capsulatum*; DK: Diffuse Karyorrhexis with mainly Extracellular *H. capsulatum*; DWOT: Died Without Treatment; GI: Granuloma Inguinale; Histo: Histomorphological Features; KS: Kaposi Sarcoma; L: left, LM: Labium Majus, LMi: Labium Minus, LN: Lymph Node, LTFU: Lost to Follow-up, M: Multiple; N: Nodules; ND: Not Documented; NHL: Non-Hodgkin Lymphoma; No: Patient Number; P: Papules; PI: Plaques; PTB: Pulmonary Tuberculosis; R: Right, Re: Resolved; S: Single; SCC: Invasive Squamous Cell Carcinoma; SI: Systemic Illness; TB: Tuberculosis, U: Ulcers; *: Morphology of initial biopsied lesion/s; #: Patient on treatment at time of second set of biopsies

Table 2: Summary of clinicopathological findings.

diagnosed and coded as histoplasmosis from 1 January 1998 to 31 December 2014; using the SNOMED word and code search engines. Clinical data, including patient age, distribution and morphology of the cutaneous lesion/s, the presence of systemic disease, HIV status, CD4 count, response to treatment and disease outcome, was extracted from departmental and patient records. Stored slides or recut and stained hematoxylin and eosin (H&E), periodic acid Schiff (PAS), Southgate mucicarmine, Fontana-Masson and Gomori Grocott methenamine silver (GGMS) stained sections were re-assessed. Cytomegalovirus (CMV) (clone: CCH2, 1:400 dilution, Dakopatts, Glostrup, Denmark), Herpes simplex virus (HSV) (polyclonal, 1:300 dilution, Dakopatts, Glostrup, Denmark) and Human herpesvirus 8-latent nuclear antigen-1 (HHV8-LNA-1) (clone: 13B10, Novocastra, Newcastle-upon-Tyne, United Kingdom) immunohistochemistry were undertaken on vulval biopsies from 1 patient each, using a microwave-based, heat-assisted antigen retrieval technique as per standard methodology. Both stains employed hematoxylin as the counterstain. The Novolink polymer detection system (Leica Microsystems, Newcastle Upon Tyne, United Kingdom) and diaminobenzidine chromogen were employed for antibody visualization.

Molecular investigation was undertaken on a cutaneous facial plaque biopsy from patient 6 (P6). Briefly, DNA was extracted using the QIAamp FFPE DNA extraction kit (Qiagen, Valencia, CA, USA) according to manufacturer's instruction (Qiagen, Valencia, CA, USA). The integrity of the extracted DNA was assessed by polymerase chain reaction (PCR) for a 309-base pair segment of the *Beta actin* gene [17]. Twenty nanograms of genomic DNA was used in each 25 µl PCR using the

FastStart Taq DNA polymerase PCR kit (Roche Bioscience, Palo Alto, CA, USA) according to manufacturer's instruction (Roche Bioscience, Palo Alto, CA, USA). PCR was performed on a CFX-96 (Bio-Rad, Hercules, CA, USA) thermal cycler. Thereafter, PCR for the 25S rRNA 3' end ETS and 18S rRNA gene of *Histoplasma capsulatum* was performed using primers; Forward 5'-CTCGAGCATCCGCGGTATC-3' and Reverse 5'-CTGGCAGAATCAACCAGGTAGCTAG-3' (Roche Bioscience, Palo Alto, CA, USA). The 439 base pair product that was generated was subsequently nucleotide sequenced by Inqaba Biotechnical Industries (Hatfield, Pretoria, South Africa).

Results

General clinical details

Six of 239 patients in the study period with DH had vulval involvement and form the patient study cohort. Seventeen incisional cutaneous biopsies, 9 vulval and 7 extravulval and an inguinal lymph node excision (Table 2) that were undertaken for diagnostic purposes, were re-reappraised. The mean patient age was 32.7 (range=22-45) years. HIV testing, following the diagnosis of cutaneous histoplasmosis, confirmed HIV seropositivity in all patients. The CD4 T-lymphocyte count ranged from 3 to 55 (mean=32.7) cells/mm³. The cutaneous lesions served as the sentinel of DH, HIV infection and AIDS in the study cohort.

Specific clinicopathological features: Patients 1 to 4 (P1 to P4)

Clinical features: While all patients presented primarily for

management of the cutaneous lesions, all were emaciated and patient 3 also complained of dyspnea. Variably tender, papular, nodular and ulcerative cutaneous lesions were widely disseminated on the scalp, neck, face, trunk, upper and lower limbs, buttock, genital and perineal skin of all patients. Three patients had multiple vulval lesions. Whereas malignancies and infections of varied origin were clinical considerations, histoplasmosis was not (Table 2).

Histopathological features: Two biopsies (patients 1, 2 and 4) and 3 biopsies (patient 3) were undertaken simultaneously from vulval and extravulval sites (Table 2). All vulval cutaneous biopsies, extravulval cutaneous biopsies from P1, P2 and P4 and the lymph node from P2 demonstrated a diffuse infiltrate of histiocytes with voluminous cytoplasm (Figure 1A) containing round to oval organisms, 2 to 4µm in diameter (Figures 1B and 1C), with perifungal clear spaces and variable narrow-necked budding, in keeping with HCVC infection. While a solitary cutaneous thigh nodule from P3 demonstrated diffuse pandermal karyorrhexis (Figure 1D) with a myriad extracellular HCVC amidst nuclear debris (Figure 1E) and transfollicular fungal elimination (Figures 1F and 1G), such karyorrhectic foci were a focal finding in the other vulval and extravulval cutaneous biopsies. Focal non-necrotizing granulomatous inflammation was evident in an extravulval cutaneous biopsy from P2. Scattered lymphocytes and plasma cells were present.

Intravascular circulating monocytes with HCVC were noted in all biopsies (Figures 2A and 2B). Intra-histiocytic fungi were noted within nerves in 1 biopsy each from the labium majus of P1 and P3 (Figure 2C). Biopsies of the vulval plaques from P1 and P3 demonstrated pseudoepitheliomatous hyperplasia and transepidermal fungal elimination (Figures 2D and 2E).

Outcome: Culture of extravulval cutaneous lesions was undertaken following histopathological diagnosis; these confirmed HCVC. Fungal yeast forms of HCVC were also confirmed in sputum samples from P3. Patient 1 was treated with amphotericin B intravenously for 3 days and was discharged on itraconazole. At follow-up 4 weeks later, there was dramatic healing of her genital and extragenital cutaneous lesions. Patients 2, 3 and 4 died within the first 3 days of admission without any treatment.

Specific clinicopathological features: Patient 5 (P5)

Clinical features: P5 had disseminated, slightly hyperpigmented cutaneous lesions (Figure 3A). Because of the varied morphological features and clinical suspicions, 1 vulval and 2 extravulval biopsies were undertaken for histopathological assessment.

Histopathological features: Vulval and extravulval cutaneous

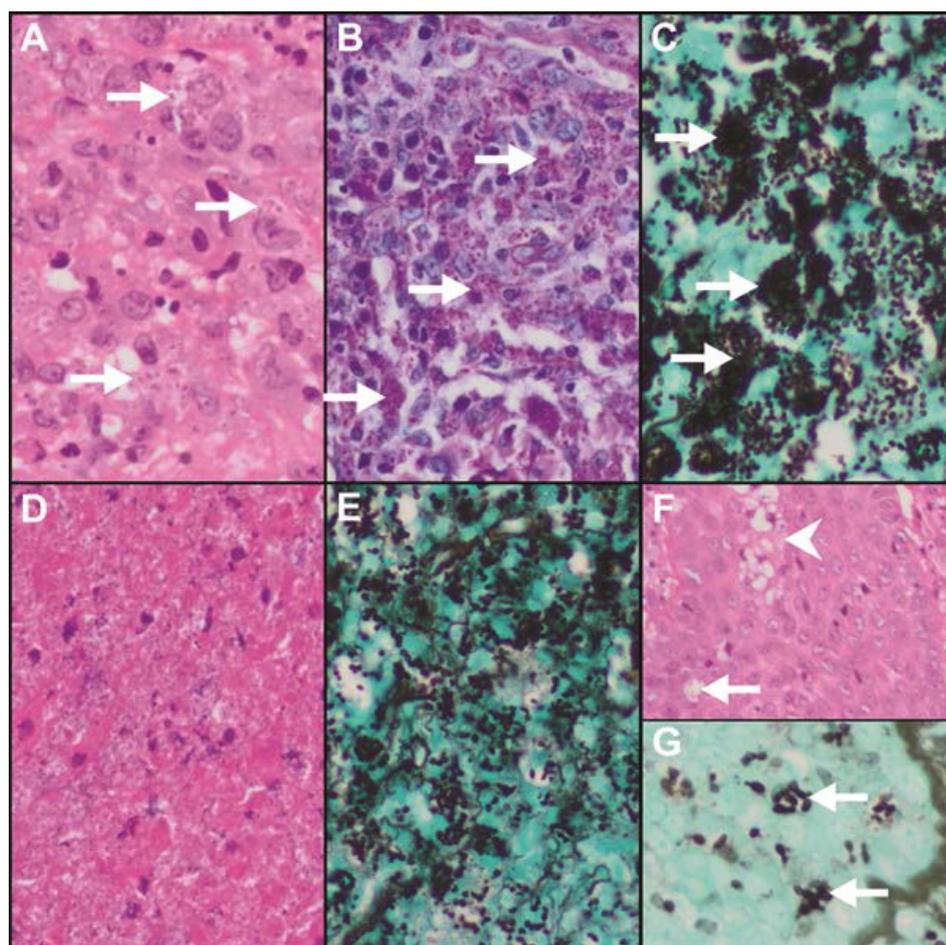


Figure 1: Cutaneous extravulval papular lesion (patient 1) with a diffuse infiltrate of histiocytes parasitized by HCVC [A, arrows (H&E 480X)], confirmed on PAS [B, arrows (480X)] and GGMS [C, arrows (480X)] stains. Cutaneous extravulval thigh nodule (patient 3) demonstrating pandermal diffuse karyorrhexis [D (H&E 480X)] with a myriad extracellular organisms [E (GGMS 120X)] and transfollicular fungal elimination [F, arrow (arrowhead=sebecytes) (H&E 480X)] highlighted by GGMS staining [G, arrows (480X)].

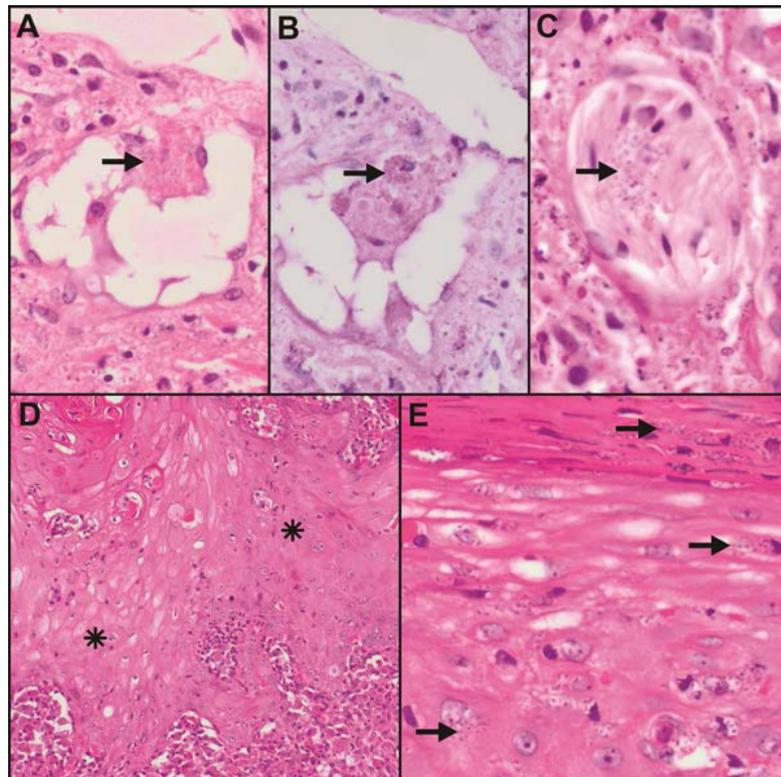


Figure 2: Vulval biopsy (patient 3) with intravascular, intrahistiocytic HCV [A, arrow (*H&E* 240X)] illuminated by PAS staining [B, arrow (240X)], intraneural organisms [C, arrow (*H&E* 240X)], pseudoepitheliomatous hyperplasia [D, asterisks (*H&E*, 480X)] and transepidermal fungal elimination [E, arrows (*H&E* 480X)].

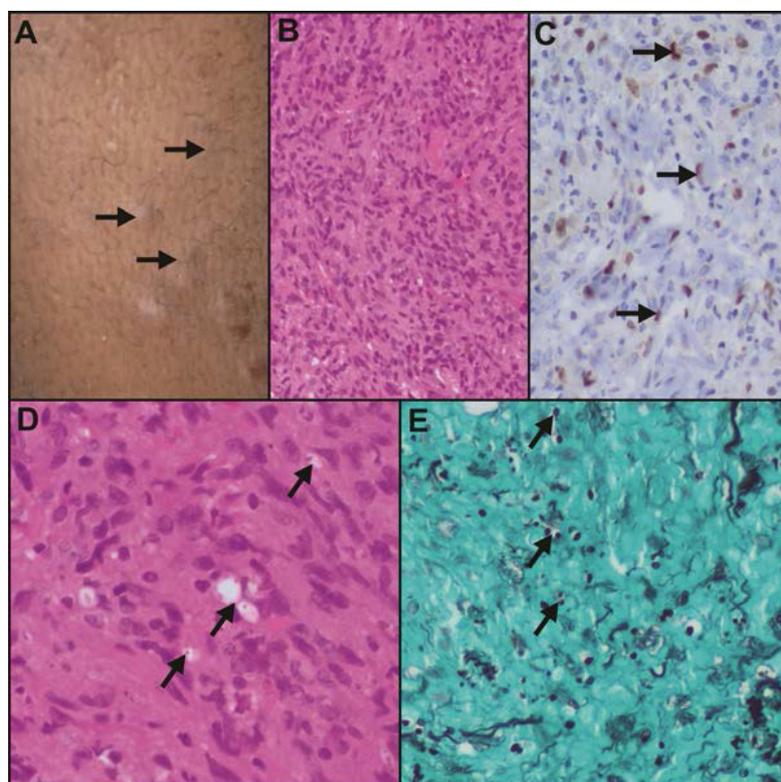


Figure 3: Patient 5 with extravulval Kaposi sarcoma [A, arrows] and vulval Kaposi sarcoma composed of malignant spindle cells [B (*H&E* 120X)]. HHV8-LNA-1 immunopositive tumour cells [C, arrows (*HHV8-LNA-1* 120X)]. Admixed histiocytes with intracytoplasmic fungi [D, arrows (*H&E* 240X)], highlighted by GGMS staining [E, arrows].

biopsies demonstrated pandermal collagen dissection by spindle cells and capillary-caliber vasculature (Figure 3B). The neoplastic cells were HHV8-LNA-1-immunopositive (Figure 3C). Hyaline globules, erythrocyte extravasation, hemosiderin pigment deposition and a lymphoplasmacytic infiltrate were evident. In addition, histiocytes containing organisms with the morphology of HCVC were admixed with the spindle cell component (Figures 3D and 3E). A diagnosis of disseminated co-lesional cutaneous Kaposi sarcoma and histoplasmosis was made.

Outcome: Culture of an extravulval cutaneous lesion, following the histopathological diagnosis, confirmed HCVC. P5 did not return for further follow-up.

Specific clinicopathological features: P6

Clinical features: Two biopsies from the neck and left labium majus were received initially. Following biopsy diagnosis, P6 received oral antifungal therapy for 7 days. There was variable resolution of most of the cutaneous lesions at her follow-up visit, but she complained of persistent pain and increase in the size of the facial plaque and vulval lesions. Biopsy of the facial and vulval plaques and of the right labium majus ulcer was then undertaken.

Histopathological features: The initial extravulval and vulval cutaneous biopsies from patient 6 (Figures 4A-4C) demonstrated a diffuse infiltrate of large histiocytes and multinucleate giant cells with 8 to 14 μm intracytoplasmic yeast forms (Figure 4A) with thick, refractile walls (Figure 4B). GGMS stains highlighted fungal chains, figure-of-8 and narrow-necked budding forms (Figure 4C). Southgate mucicarmine and Fontana-Masson stains were negative. These features were compatible with HCVD and online bioinformatics investigations [18] of the PCR-generated fragment confirmed the same.

Subsequent biopsy of the persistent right labium majus and facial plaques demonstrated variable ulceration and pseudoepitheliomatous hyperplasia with transepidermal fungal elimination, dermal fibrosis and admixed HCVD-laden histiocytic aggregates, scattered neutrophils, lymphocytes and plasma cells. In addition, biopsy of the plaque in the right labium majus also demonstrated intranuclear owl's eye and amphophilic cytoplasmic inclusions of CMV in endothelial cells of thrombosed vasculature, histiocytes, eccrine units and myofibroblasts (Figures 5A and 5B). The superficial shave biopsy of the left labium majus ulcer demonstrated HSV inclusions within the nucleus of keratinocytes, some of which were multinucleate (Figures 5C and 5D).

Outcome: Aciclovir and ganciclovir were added to the patient's therapeutic regimen. Resolution of the skin lesions was confirmed at 6 weeks. Biopsy of a uterine cervical mass 6 months later confirmed an invasive squamous cell carcinoma. She was lost to further follow-up.

Discussion

DH occurs predominantly in individuals with compromised immunity or with a massive inoculum [12]. Classical histoplasmosis, documented in 1 in 1000 to 1 in 500000 people in the pre-AIDS era, mainly in transplant recipients, in patients on immunosuppressive therapy, with hematologic malignancies, inborn errors of immunity or at the extremes of age [4,5,12], is acquired by inhalation of spores from soil contaminated by bird and bat guano [4,12]. Patients with AIDS and low CD4 counts, however, are at greatest risk for DH, documented as the first presentation of opportunistic infections in 75% of patients with AIDS [8]. Cutaneous lesions occur in approximately 50% of AIDS patients with disseminated classical histoplasmosis [3]. In contrast to classical histoplasmosis, the ecological and pathogenetic profile

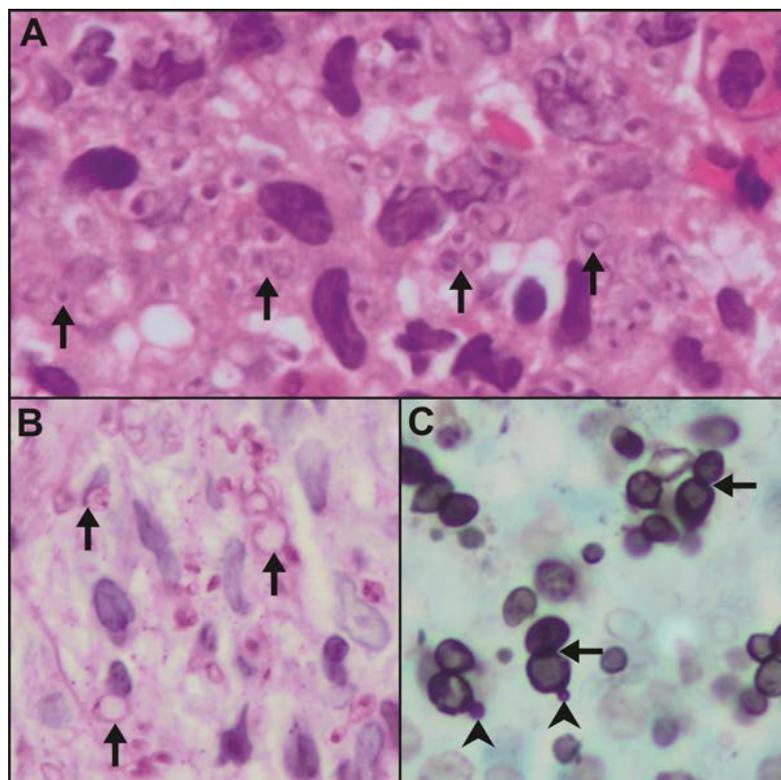


Figure 4: Vulval biopsy from patient 6: Transdermal and subcutaneous HCVD [A, arrows (H&E)] with prominent capsules [B, arrows (PAS oil immersion 1200X)] and figure-of-8 morphology [C, arrows (GGMS oil immersion 1200X)] and narrow-necked budding [C, arrowheads].

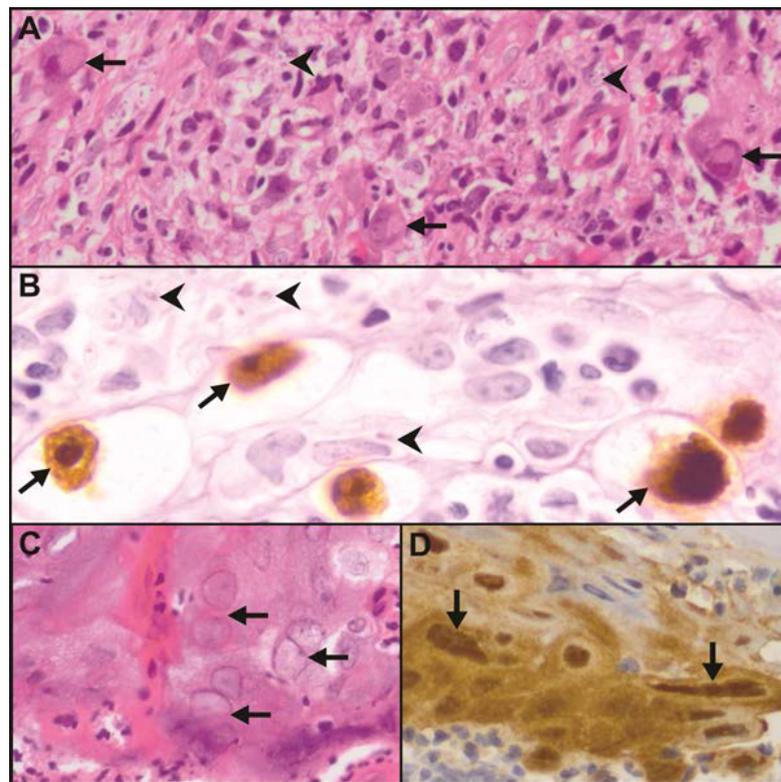


Figure 5: Vulval biopsies from patient 6: CMV inclusions within endothelial cells [A, arrows (H&E 240X)], confirmed immunohistochemically [B, arrows (CMV oil immersion 1200X)] and surrounding HCVD [B, arrowheads]. Intranuclear HSV inclusions in keratinocytes [C, arrows (H&E 480X)], confirmed immunohistochemically [D, arrows (HSV, 480X)].

of African histoplasmosis, first described in 1952 [19] and endemic in West and Central Africa and Madagascar, is poorly understood [1]. Classical histoplasmosis is typified by predominant pulmonary involvement while African histoplasmosis is characterized mainly by cutaneous, subcutaneous and osseous disease [19]. The cutaneous lesions, however, comprise a spectrum of manifestations, including papules, pustules, nodules, ulcers, acneiform lesions, verrucous plaques, vesicles, purpura and erythema multiforme-like lesions [1-4,8,19]. Because of the clinical heterogeneity, the definitive diagnosis of classical and African histoplasmosis requires culture and fungal isolation from clinical secretions or biopsy samples [20].

While genital histoplasmosis is not uncommon in males, female genital tract histoplasmosis has been documented rarely in the English-language literature (Table 1) [9-15]. Of these, 1 patient each had ovarian, vulval, vaginal and cervical histoplasmosis and 2 had vulvovaginal histoplasmosis. Two of the 6 reported patients with cervical and vulvovaginal histoplasmosis were HIV-positive. It is surprising that vulval cutaneous involvement in patients with DH is documented rarely in patients with AIDS and DH. Whether this represents a true rarity or an under-reporting phenomenon is uncertain. It is possible, however, that when patients with DH and lesions in exposed skin are in an ill state, little attention is paid to lesions in unexposed sites. Hence, the rarity of vulval disease may be a function of non-reporting rather than true non-occurrence. In the present study, while all patients sought medical attention for disseminated cutaneous lesions, the vulval lesions were biopsied because the patients complained of pain therein, a poor response to therapy or to exclude potential dual co-morbid pathology in genital and extragenital locations. The latter indication

arose because the vulval lesions were not distinctive clinically, and the clinical suspicions included infections such as tuberculosis, granuloma inguinale and syphilis and malignancies that included squamous cell carcinoma and Kaposi sarcoma (Table 2). The vulval lesion of patient 5 was biopsied to confirm the clinical suspicion of Kaposi sarcoma. Concomitant co-lesional Kaposi sarcoma and histoplasmosis has been diagnosed once in the literature [21], but such co-occurrence is unreported in the vulva. Less attention was paid to the genital disease by P6 and the attending clinician at the initial visit. The vulval lesions were re-biopsied because of increasing lesional pain and the suboptimal response of the vulval ulcers and plaque to initial antifungal therapy. Hence, vulval biopsies were important to distinguish histoplasmosis from other vulval infections and tumors and to confirm the co-existence of co-morbid infective and neoplastic diseases.

The well-known histopathological patterns of DH include diffuse dermal histiocytic infiltration, dermal karyorrhexis and necrotizing and non-necrotizing granulomatous inflammation [2-5,8]. More recently, lichenoid, nodular pseudomyxoid, pyogenic granuloma-like, perifollicular, superficial, mid and deep dermatitic, focal nodular dermatitic and histiocytic lobular panniculitic patterns have been documented in AIDS-associated DH [22]. The clinical spectrum of cutaneous VH in the present study was similar to that commonly documented in the literature, including ulcers, papules, papulonodules and plaques [4,5,8]. The cutaneous histopathological spectrum was limited to diffuse, pandermal histiocytic infiltration, diffuse, pandermal karyorrhexis and focal non-necrotizing granulomatous inflammation. Histoplasmosis was not suspected as a cause of the vulval disease clinically but a suspicion of sexually transmitted diseases and

malignancies prompted vulval biopsies. Whether the painful nature of the genital lesions was a function of the rich neural supply of the vulval/perineal region or whether it is a clinical reflection of *H. capsulatum* invasion of the nerve plexus is uncertain, as is the contributory role of the viral co-infections in the pathogenesis of pain. Notwithstanding this uncertainty, it is important to recognize that the painful nature of the genital lesions prompted vulval biopsies and diagnosis of VH in all patients, in addition to co-lesional, co-morbid diseases in 2 patients. In 4 patients DH represented advanced fatal disease. While long term follow-up was unavailable for 2 patients, it is noteworthy that despite their profound immunocompromise, their cutaneous lesions responded to appropriate, timely therapy.

Because of the non-specific nature of the cutaneous disease, histopathological, mycological, serological and molecular tests are helpful in definitive diagnosis [23,24]. While fungal culture has been the gold standard for the diagnosis of histoplasmosis, the diagnostic technique is disadvantaged by the slow growth characteristics of *H. capsulatum* and the challenges of laboratory-based temperature-dependent mycelium-to-yeast transformation [24]. *H. capsulatum* antigen tests may be positive in 92% and 83% of urine and serum samples from patients with AIDS, respectively, but fungal cross reactivity and immunocompromise-related negativity may produce incorrect results [19,24]. Histopathological assessment not only facilitates rapid diagnosis of histoplasmosis but also enables confirmation of co-lesional co-infections, as was present in 2 vulval biopsies from patient 4. As in P6, confirmation of histoplasmosis is also possible from wax-embedded tissue using PCR [24].

The complete careful appraisal of genital tract lesions with a granulomatous, diffuse histiocytic and karyorrhctic background with optimally undertaken special stains for infections, are invaluable in overcoming the mimicry that is associated with the infections that may involve the vulva. These special stains include Ziehl Neelsen, Fite-Faraco, PAS, GGMS, Warthin-Starry and Giemsa. As evidenced in this report, a range of infective and non-infective diseases may mimic the histopathological spectrum of VH. The recognition of the 2-4 µm budding yeast forms is the gold standard for diagnosis of HCVC [1,4,5]. The presence of granulomas with or without necrosis may mimic secondary or tertiary syphilis, tuberculosis, cryptococcosis and leishmaniasis. Secondary syphilis may be accompanied by epidermal alterations including a lichenoid or psoriasiform phenotype and endothelial cell swelling. When present, a dense plasma cell infiltrate facilitates the diagnosis [25]. The recognition of spirochetes using silver stains and *T. pallidum* by immunohistochemistry is helpful for microscopic confirmation, but partially treated syphilis may pose diagnostic challenges. The role of PCR for *T. pallidum* confirmation in formalin-fixed paraffin-embedded tissue and correlation with treponemal serology cannot be overvalued in such settings. While *C. neoformans* is uncommon in the female genital tract, the identification of *C. neoformans* may be a challenge in paucifungal granulomatous lesions and when capsule-deficient *C. neoformans* predominates. In the former, use of mucicarmine stains will be helpful and in the latter, Fontana Masson staining will differentiate *C. neoformans* from *H. capsulatum* [26]. The smaller size of leishmanial organisms and the identification of the kinetoplast on Giemsa-stained sections distinguish leishmaniasis from histoplasmosis [25,27]. Fite-Faraco stains may assist the identification of acid fast bacilli in tuberculoid leprosy [28], especially in nerves, but mycobacterial PCR studies may be necessary to demonstrate a definitive *M. leprae* origin.

Biopsies with a diffuse histiocytic infiltrate containing “parasitized”

H. capsulatum mimic leishmaniasis, lepromatous leprosy, penicilliosis and granuloma inguinale. The first 2 infective agents are smaller than HCVC and the Giemsa and Fite-Faraco special stains are invaluable in confirming each one, respectively [4,25]. The presence of septate yeasts is useful in differentiation of *P. marneffei* from HCVC yeasts with narrow-necked budding [29] especially as these infective agents have overlapping clinical manifestations, laboratory findings and chest radiographic abnormalities [30]. While Donovan bodies of granuloma inguinale may mimic HCVC, compelling clues to granuloma inguinale include the plasma cell background with microabscesses, absence of budding organisms and smaller size of the causative agent, *Klebsiella granulomatis* [25].

Because of the larger size and shape characteristics of HCVC, cutaneous cryptococcosis, penicilliosis and blastomycosis may pose diagnostic challenges [31]. *C. neoformans* may also grow in chains but the carminophilous capsule sets it apart from HCVC [25,26]. *P. marneffei* demonstrates distinct septation and *B. dermatitidis* has multiple nuclei and broad-based budding [31]. Lastly, biopsies with karyorrhctic foci may mimic non-infective, inflammatory conditions including lupus erythematosus, Sweet syndrome and vasculitis, but special fungal stains are diagnostic. While the clinical features of these patients may be helpful, careful attention to the variation in size of nuclear fragments and the presence of fungal forms with distinctive, well-defined contours on silver stains underpins the diagnosis of an infective process.

In conclusion, vulval involvement as part of DH is eminently treatable. The clinical morphological features of vulval involvement by DH are not distinctive. VH shares overlapping clinical features with a range of infections and tumors. Hence, biopsy of vulval lesions is pivotal, not only for diagnosis and monitoring of a therapeutic response, but also, as noted in this study, for the recognition of co-infective pathology and malignancy. The spectrum of cutaneous disease, including vulval involvement, served not only as the initial clue to DH, but also as a sentinel of HIV infection, AIDS, Kaposi sarcoma and CMV and HSV co-infections in the study patients.

Acknowledgement

We thank Mrs. M Moodley for manuscript word processing, formatting and assistance with literature access and Mr. Dinesh Sookhdeo for patient data collation.

Declaration

Permission to undertake this study was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (Reference Number BCA535/16).

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