HISTOPATHOLOGICAL DIAGNOSIS OF SUBCUTANEOUS *DIROFILARIA REPENS* INFECTION IN HUMANS

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Abstract. The host tissue response in humans, based on a study of biopsies of 14 subcutaneous nodules caused by *Dirofilaria repens* is described. The response was characterized by accumulation of eosinophilic material or eosinophilic pus adjacent to the parasite with surrounding granulomatous inflammation associated with extensive eosinophil infiltration of the surrounding tissue. Unlike with fungal lesions, fat necrosis was conspicuously absent. Nodules were all less than 3 cm in size. Fifteen percent of the smaller nodules (less than 1 cm) and 30% of the larger nodules required extensive examination of tissue to visualize the parasite. It is concluded that when typical tissue reactions are seen, extended histological processing is indicated for accurate diagnosis of dirofilariasis, by demonstrating the parasite.

INTRODUCTION

Dirofilariasis, is a zoonotic infection, which could be caused by several species of filarial worms of the genus *Dirofilaria*. Of these, *D. repens*, a common filarial worm of dogs and cats in the old world, accounts for the most number of human infections with over 400 cases recorded (Pampiglione *et al*, 1995). Human infection is common in several countries. In Sri Lanka, infection was first recorded in 1962 but with increased awareness, to date over 80 cases are documented (Dissanaike *et al*, 1997) and currently Sri Lanka is one of the countries showing a high prevalence of this infection. Dirofilariasis is very common in dogs in Sri Lanka with infection rates varying from 30-60% (Dissanaike, 1961; Dissanaike *et al*, 1997). Furthermore, several species of common man biting mosquitoes have been shown to be efficient vectors of this parasite (Dissanaike *et al*, 1997). Thus it is highly likely that infection is still underreported.

Clinically, infection usually presents as a subcutaneous nodule, which is invariably excised for a histopathological diagnosis. Confirmation is not a problem if the excised nodule shows a dead or a live worm, or if the worm is seen in section. However, if the tissue is not thoroughly sectioned or sampled, it is possible to miss the presence of the adult worm, thereby leading to an inaccurate diagnosis. Brief descriptions of the tissue reactions to *D. repens* are documented (Billups *et al*, 1980; Beaver *et al*, 1987; Pampiglione *et al*, 1995; Shekhar *et al*, 1996). However, these descriptions are not sufficiently specific or adequate to facilitate a diagnosis. The aim of this study was (1) to characterize the tissue reactions in humans to subcutaneous *D. repens* infection, and (2) to document the amount of tissue that needs to be examined, in order to visualize the parasite in histopathological preparations.

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MATERIALS AND METHODS

The study was based on biopsy material from 14 subcutaneous nodules caused by *D. repens*, all of which showed the presence of the adult parasite. The excised nodules had been fixed in 10% formol saline, and 5 μm thick paraffin sections stained with hematoxylin and eosin were examined for the histopathological diagnosis. Identification of the worm was based on, the maximum width, characteristic cuticle with sharp longitudinal cuticular ridges, and thick body wall with prominent lateral chords and tall musculature. The dimensions of the excised nodules after formalin fixation were noted. The cases requiring deeper cuts of the original blocks, or those needing further examination of the excised material for the parasite to be visualized, were also noted.

RESULTS

The clinical profile of the patients, size of the post fixed lump and parasite morphometry, are shown in Table 1:

(a) Tissue reaction immediately adjacent to the parasite

In 13 cases, the worm showed varying grades of degeneration, often being well preserved in one

Table 1
Clinico-parasitological profile of subcutaneous dirofilariasis.

No	Age (Years)	Sex	Location	Size of post-fixed lump in cm	Parasite morphology	
					Maximum width in μm	description
1	16	M	abdominal wall	2 x 2 x 0.5	280	Degenerate
2	41	F	thigh	2 x 1.5	390	Mature female
3	65	F	cheek	1.2 x 0.8	450	Degenerate
4	48	M	back of chest	1.5 x 0.5	220	Immature male
5	45	F	breast	1 x 0.5	250	Anterior region of worm
6	5	M	conjunctiva	0.5 x 0.5	320	Live worm, male
7	16	M	forearm	0.5 x 0.5	310	Female
8	30	M	back of chest	1.5 x 1	350	Mature female
9	40	M	temporal region	1 x 0.8	210	Degenerate
10	34	F	forehead	0.5 x 1	280	Female
11	22	F	anterior chest	2 x 1.5	430	Mature female
12	1.5	M	scrotum	1 x 0.5	190	Female
13	45	M	cheek	2.5 x 1.5	420	Mature female
14	1.5	M	scrotum	0.5 x 1	250	Male

area and poorly preserved in other areas. The worm was surrounded either by fibrinoid watery eosinophilic material, densely eosinophilic Splendore-Heoppli like material or eosinophil pus formed by necrotic and degenerate eosinophils and other inflammatory cells (Fig 1). Caseous type necrotic material was seen in one case. In the case where a live worm was extracted from the lump at the time of surgery, the cavity in which the worm lay was lined by fibrinoid material without degenerate inflammatory cells. An epithelioid granulomatous reaction associated with multinucleate giant cells was seen in five cases. Coexistence of a granulomatous reaction, and the eosinophilic substances mentioned above were noted. The granulomata resembled those seen in mycobacterial and fungal infections. Occasionally, foreign body types multinucleate giant cells were seen adherent to the cuticle of the worm.

(b) Tissue reaction further away from the parasite

The tissue plane in which the parasite was located was the subcutaneous tissue and consisted of loose connective tissue and the subcutaneous fat. The tissue in all instances was heavily infiltrated with eosinophils. Varying numbers of lymphocytes, neutrophils and plasma cells were seen. Lymphocytes were some times seen to form large aggregates and follicles with germinal centers and in one case was seen to infiltrate the connective tissue in an Indian file pattern mimicking a lymphoma. Neutrophils were prominent in only one case and were usually inconspicuous. In the cases where the parasite was surrounded by necrotic tissue, granulation tissue heavily infiltrated with eosinophils was seen at the periphery.

Similarly, the tuberculoid granulomata, were surrounded by granulation tissue rich in eosinophils. One case showed marked fibrosis of the tissue, and the parasite was calcified in several areas. The subcutaneous fat showed a septal and lobular inflammation mediated by eosinophils and lymphocytes. Fat necrosis of adipocytes, the presence of foam cells and Touton giant cells, and the formation of oil cysts, all of which are seen in fat necrosis, were conspicuously absent (Fig 2). The underlying skeletal muscle, when included in the excised material, showed severe inflammation mediated by eosinophils and lymphocytes. with destruction of muscle fibers.

The size of the excised nodule usually varied between 0.5 and 1.5 cm in diameter. Occasionally larger lumps were excised (Table 1). The tissue measuring between 0.5 cm and 1 cm, were processed fully in the first instance, in one or two blocks. Of these, 85% showed the parasite in the initial sections. The remaining 15% required step sectioning of the blocks, for the parasite to be visualized. The tissue measuring over 1 cm, were processed in part initially, and 70% of these showed the parasite in the initial sections. The remaining 30% required step

Fig 1–Parasite in a pool of eosinophilic material (x 60).

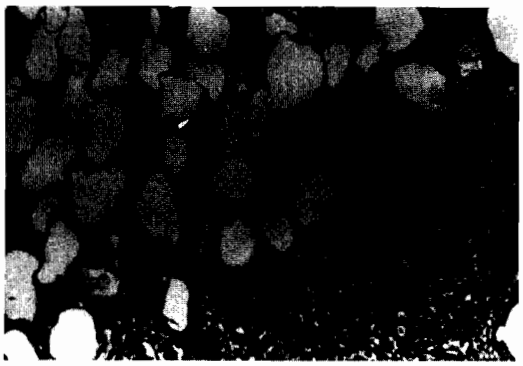

Fig 2–Dirofilarial lesion in the subcutaneous fat showing infiltration with eosinophils. Fat necrosis is not evident (x 120).

sectioning or processing of the entire sample for the parasite to be visualized. In the cases requiring step sectioning or processing of further tissue, identical tissue reactions to those described above were seen, in the absence of the parasite.

DISCUSSION

Lesions caused by *D. repens* present usually as subcutaneous nodules, at a variety of locations and are seen in all age groups in Sri Lanka (Dissanaike *et al*, 1997) . Although subcutaneous lesions are the common clinical presentation, deep seated lesions caused by this worm have also been documented (Pampiglione *et al*, 1995). Rim (1976), reported an infection in the peritoneal cavity where the patient had very likely acquired the infection in Sri Lanka. This study shows that several types of tissue reactions occur in these lesions. Immediately adjacent to the parasite, accumulation of fibrinoid material and or accumulation of pus derived from eosinophils were noted. Deposition of similar fibrinoid material has been reported by Billups *et al*, (1980). Tuberculoid type of granulomatous inflammation, and formation of granulation tissue, all of which are accompanied by the presence of eosinophils, were additional findings. Lichtenberg (1987) and Jungmann *et al* (1991) described very similar tissue changes in association with bancroftian filariasis. However, none of these studies describe caseous type necrosis in filarial lesions, which we observed in one case. Although mycobacterial and fungal infections of the skin too can elicit similar reactions, the presence of eosinophils should favor a suspicion of a parasitic infection in the event of the parasite being absent in the sections. In the latter event, the characteristic tissue reaction should prompt examination of further sections or more extensive tissue sampling, which would then reveal the parasite in most cases. Although rare, sparganosis, a parasitic zoonosis caused by the larval stage of *Spirometra* sp is yet another parasitic infection, which could cause similar lesions (Wijesundera *et al*, 1997). It should be noted that the non specific condition of eosinophilic panniculitis also gives an identical tissue reaction (Mckee, 1989). In this study it has been shown that 15% of specimens measuring 1 cm or less, and 30% of larger specimens, required step sectioning or processing of additional tissue for the parasite to be detected. Clearly inadequate sampling of excised material would prevent an accurate diagnosis. The absence of fat necrosis in the presence of septal inflammation in the adipose tissue mediated by eosinophils and lymphocytes, is an important observation. Subcutaneous fungal infections which also could show an eosinophil mediated inflammation are usually associated with fat necrosis of the adipose tissue (Mckee, 1989). This could be a useful differentiating feature between dirofilarial and fungal infections. The inflammatory process was seen to extend widely even to adjacent skeletal muscle tissue. Both dead and live worms were associated with similar tissue reactions and the state of preservation of the parasite did not appear to influence the nature of the tissue reaction. Wartmann (1944) made similar observations in lymphatic filariasis. The lesion showing much fibrous tissue, and partially calcified worm was probably an old lesion. It is noteworthy that even this lesion showed the characteristic tissue reaction.

CONCLUSIONS

Eosinophilic material, eosinophilic pus, granulomatous inflammation and the formation of granu-

lation tissue, all in association with a heavy infiltrate of eosinophils, is seen in association with *D. repens* infection of the subcutaneous tissue in humans. Inflammation of the adipose tissue is not associated with fat necrosis. This type of tissue reaction in the subcutaneous tissue should alert one to the possibility of parasitic infections, when a worm is not seen on initial sections. Use of specific DNA probes in tissue sections would undoubtedly facilitate further the diagnosis of dirofilariasis.

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