

## Clinicopathological Study of Subcutaneous Heminthcnodules : Unusual Sites and Diagnostic Dilemmas

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Superficial palpable swellings with helminthic infection as an underlying etiology is usually an accidental finding in the surgically excised specimens. Somatic nematodes and cestodes are the commonly implicated organisms, and the zoonotic nematodes show an emerging trend. The present study aims to reappraise the histopathological findings of helminthic etiology in superficial swellings which were clinically suspected to be of neoplastic/non neoplastic nature. Thirty six cases of palpable superficial nodules with infective etiology over a period of five years were reviewed. 19/36 were of helminthic etiology were included in the present study. Pertinent demographic and clinical data were retrieved from the medical archives. Amongst the 19 cases, 8 were males and 11 females. Chest wall (4/19), and eyelids (3/19) were the most common sites involved. The size ranged from 0.8-15 cm in greatest dimension. Presence of histiocytes (13/19), granulomas (11/19), eosinophils (10/19), and giant cells (9/19) were the most consistent histological findings. 14 cases had discernible parasite morphology with diagnosis of filarial worms (7/19), *Dirofilaria* (3/19), cysticercosis (4/19), and hydatid cyst (1/19). Four cases had dead and calcified parasites with no discernible morphology. Granulomatous inflammation and tissue eosinophilia are strong indicators of a parasitic etiology. Subcutaneous and intramuscular filariasis, cysticercosis and hydatid cyst are well documented etiologies whereas *Dirofilaria* is an emerging zoonotic infection with worldwide case reports. Imaging techniques and fine needle aspiration can point towards the diagnosis; however in the absence of characteristic features, histopathology can be relied upon to diagnose a helminthic etiology.

**Keywords:** Cysticercosis; *Dirofilaria*; *Filaria*; Granulomas; Zoonoses.

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Superficial skin/ subcutaneous nodules can have varied neoplastic or non-neoplastic aetiologies. These usually include tumors, skin appendage lesions, tumor-like lesions, inflammatory and infective lesions<sup>1</sup>. A helminthic infection as an underlying etiology is often

an accidental finding in the surgically excised specimens of these swellings. Due to increased international travel, immigration, climate changes, and iatrogenic immunosuppression, uncommon infections like fungal, protozoan, helminthic and ectoparasites are now reported with increasing

frequency<sup>2</sup>. Somatic nematodes and cestodes are the commonly implicated amongst the helminthic agents, and the zoonotic nematodes show an emerging trend with increasing number of case reports in literature<sup>3</sup>.

*Wuchereria bancrofti*, *Brugia malayi*, and cysticercosis is responsible for most of the cases in India<sup>4,5,6</sup>. Recently there has been an increase in subcutaneous nodules caused by *Dirofilaria* seen in various countries which was considered to be a zoonotic infection earlier<sup>7</sup>.

The present study aims to evaluate and highlight the aetiological agents and the tissue diagnostic features of superficial lesions of helminthic etiology.

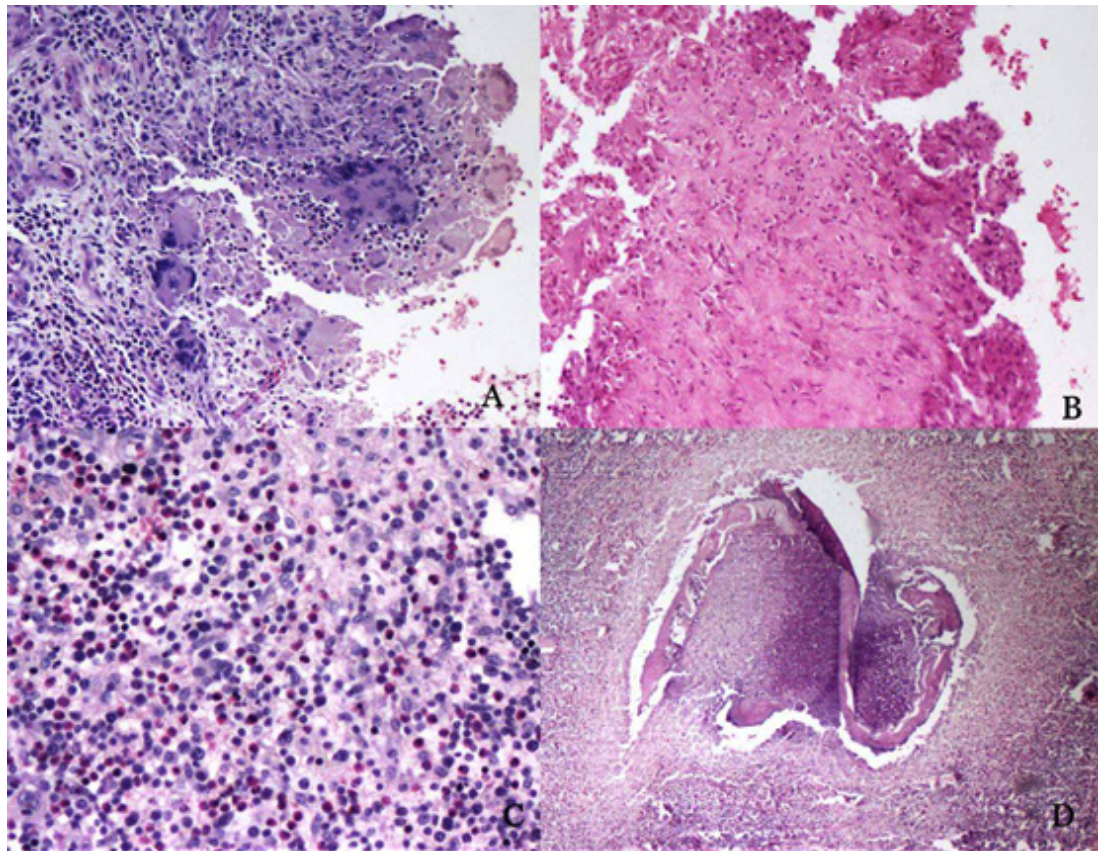
### MATERIALS AND METHODS

This was a single institution, retrospective, observational study undertaken in the department

of pathology of a tertiary care center over a period of five years (2012-2017). Thirty-six cases of surgically excised palpable superficial nodules unsuspecting a parasitic etiology were reviewed. Out of the 36, 19 cases diagnosed as having helminthic etiology were included in the study. Electronic archives and test requisition forms were used to collect pertinent demographic and clinical data including the age, sex, clinical diagnosis, site and size of the lesions. Hematoxylin and eosin (H&E) stained slides of the cases were retrieved, and histopathological findings were reviewed.

### RESULTS

Amongst the 19 cases included in the study, 8 were males, and 11 were females (Age - Range 1-64) with a mean age of 36.36 years. Chest wall (4/19, 21%), and eyelids (3/19, 15.7%) were the most common sites involved. Forehead, neck,



**Fig. 1.** Tissue reaction patterns showing foreign body granulomatous reaction (A, H&E, 100x), histiocytic infiltrates (B, H&E, 100x), eosinophils (C, H&E, 200x), and a calcified filarial worm with surrounding necrosis and chronic inflammation (D, H&E, 50x)

**Table 1.** Demographic and clinical details

Case	Age (years)	Sex	Site	Size (cm)	Clinical diagnosis	Histopathology diagnosis
1	35	F	Forehead	2x1	Sebaceous cyst	Dirofilariasis
2	64	F	Forehead	2.5x2	Sebaceous cyst	Filariasis
3	42	M	Neck	2.5x1.5	Lymphadenitis	Abscess with dead filarial worm
4	5	M	Right leg	1.5x1	Cellulitis	Abscessfilariasis with
5	40	M	Chest wall	4x1.5	Myositis	Filariasis
6	25	M	Inguinal region	2x1.5	Lymphadenitis	Lymphadenitis withdead filarial worm
7	38	F	Eyelid	0.8x0.5	? Tumor	Dirofilariasis
8	44	F	Abdominal wall	4x3.5	Dermoid cyst	Cysticercosis
9	23	M	Chest wall	1.5x0.6	? Neoplastic	Cysticercosis
10	26	M	Chest wall	3.5x2.7	? Neoplasm	Cysticercosis
11	52	F	Right thigh	11x7	Soft tumour tissue	Hydatidosis
12	64	F	Right thigh	15x9	Soft sarcoma tissue	Cysticercosis
13	46	F	Left eye	1.5x1	Epidermoid cyst	Abscess with dead filarial worm
14	52	F	Chest wall	1.5x1	? Neoplasm	Filariasis
15	16	F	Neck	1.5x1	Lymphadenitis	Granulomatous inflammation with dead filarial worm
16	49	F	Axilla	3x3	Lymphadenitis	Granulomatous inflammation with filariasis
17	1	M	Eyelid (lower)	1x1	Epidermoid cyst	Dirofilaria
18	33	M	Back	2x2	Lipoma	Filariasis
19	36	F	Breast	1.7x1	? Carcinoma	Filariasis

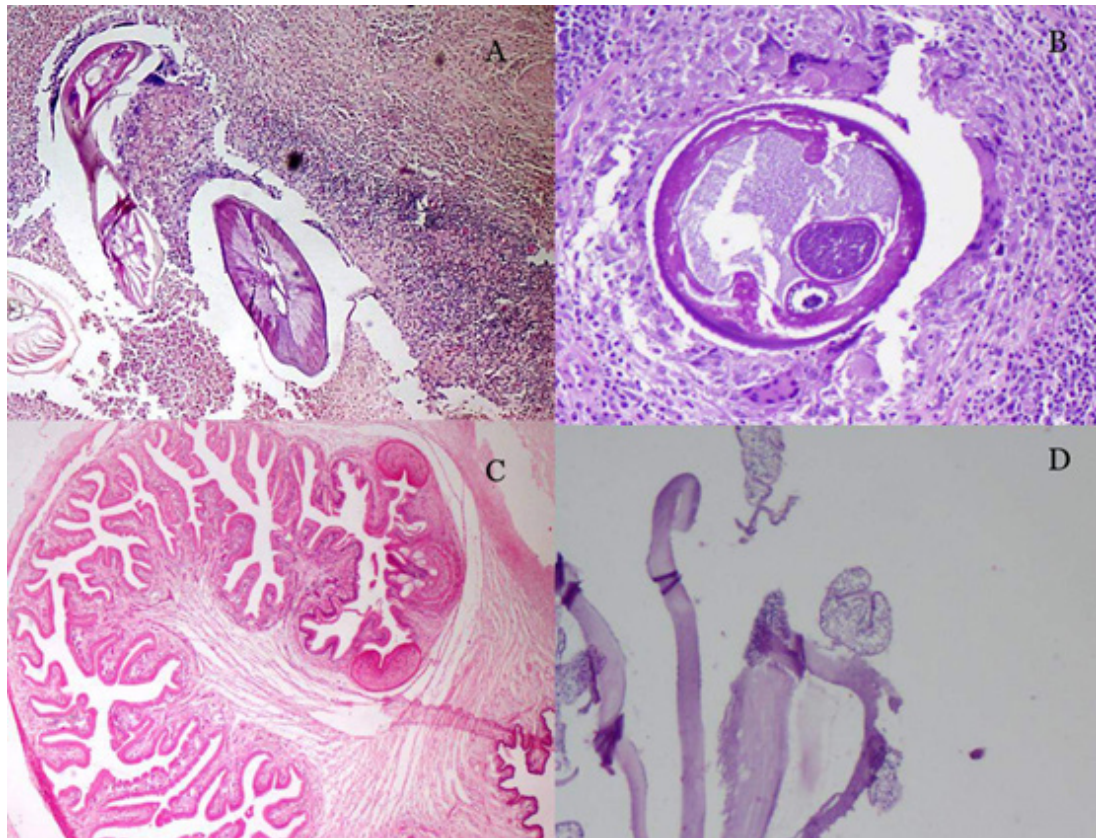
and thigh had 2 cases each (2/19, 10.5%), while axilla, abdominal wall, leg, inguinal region, breast, and back had 1 case each (1/19, 5.2%). The size ranged from 0.8 to 15 cm in the greatest dimension.

The clinical suspicion was neoplastic in 7/19 cases. Lymphadenitis was second most

common in 4/19 patients followed by an epidermoid cyst in 3/19 patients, one each of sebaceous cyst, dermoid cyst, forehead cyst, cellulitis, and myositis. In none of the cases, an infective etiology was considered as a diagnostic possibility. The demographic details with clinical diagnosis are summarised in Table 1.

**Table 2.** Histopathological findings and the associated organism

Histopathology	Filariasis	Dirofilariasis	Cysticercosis	Hydatidosis	Deadcalcified worms	Cases (%)
Indentifiableparasites	7	3	4	1		15
Histiocyticinfiltrate	6	2	2		3	13
Granuloma	4	3	1		3	11
Eosinophils	4	1	2		4	11
Giant cells	2	2	2		3	9
Chronicinflammation	4	1	2	1	2	10
Acuteinflammation	2	2		1	2	7
Necrosis	2	1	1		3	7



**Fig. 2.** (A) Cross section of a filarial parasite with surrounding necrosis, and chronic inflammatory infiltrate (H&E, 100x). (B) Cross section of dirofilaria with surrounding foreign body granulomatous reaction (H&E, 200x). (C) Cysticercus larval form in the muscle tissue, the head shows protoscolexes (H&E, 100x). (D) Hydatid sand with few fragments of the laminated wall and occasional scolices (H&E, 100x)

A characteristic parasite morphology was seen in 15 cases (78.9%), while four cases showed dead or calcified parasites on extensive examination of the tissue. Amongst the other histopathological features, presence of histiocytes (13/19, 68.4%), granulomas (11/19, 57.8%), eosinophilia (10/19, 52.6%) were the top three histopathological findings. These findings were also consistently seen in the cases without a discernible parasite in the sections (Figure 1). Complete findings and the associated agents are summarised in Table 2.

The most common etiological agent seen in the histopathology specimens was filarial worms (7/19, 36.8%) followed by cysticercosis (4/19, 21%), dirofilaria (3/19, 15.7%) and echinococcus (1/19, 5%) (Figure 2). 4/19 cases (21.1%) did not have a discernible parasite morphology, but a calcified remnant which was suspicious of a parasitic etiology.

## DISCUSSION

A variety of arthropods, protozoa, and helminths infect the skin and subcutaneous tissues and can be identified by pathologists in cytology and histology preparations. The specific organisms depend on patient's exposure history. Arthropods are the most common parasite seen and include *Sarcoptes scabiei*, *Demodex* species and *Tunga penetrans*. Helminths are less often seen, and include round worms (eg, *Dirofilaria* spp.), tapeworms (eg, *Taenia solium*, *Spirometra* spp.), and flukes (eg, *Schistosoma* spp.)<sup>8,9,10</sup>.

Filariasis is usually caused by nematodes including *Wucheria bancrofti*, *Brugia* spp. And manifests primary as lymphedema of the extremities, genitalia, and breasts. The most common presentations of *W. bancrofti* infestation are elephantiasis, chronic lymphedema, epididymitis, funiculitis, and lymphadenitis. Subcutaneous swelling is an extremely uncommon presentation of bancroftian filariasis even in endemic areas<sup>11</sup>. In contrast to *W. bancrofti*, *Loa loa* commonly presents as a subcutaneous nodule. The cycle starts when a female *Chrysops* takes microfilariae from the blood of an infected individual during a blood meal. Then the microfilariae mature toward infective larvae (L3), which become infective and can be transmitted to another human during the next blood meal. In

humans, filarial worms will develop to adult stage and then can produce microfilariae, which can be transmitted to the next individual during another blood meal. The microfilariae have a diurnal periodicity, appearing in the peripheral blood in the day time, and reach their maximum at around midday (11:00 am to 1:00 pm)<sup>12</sup> The diagnosis of filariasis is by a demonstration of microfilaria in stained or unstained blood films, circulating filarial antigen detection and demonstration of organism in histopathological sections<sup>13</sup>. Other histological features seen with filariasis are foreign body giant cells, forming tubercle-like nodules. While intact microfilariae are not generally seen, there may be fragments of the parasite in the granulomata. The eosinophilic debris, in the form of Splendore-Hoeppli material can be seen surrounding the parasite<sup>14</sup>. The inflammatory reaction develops in nodular fashion around fragmented and necrotic worms. Epithelioid cells and foreign body giant cells appear subsequently. In the present study seven cases with filariasis was seen. Tissue histiocytic infiltrate (6/7 cases), granulomas (4/7 cases), and eosinophilia (4/7 cases) were the most consistent findings. Amongst the four cases with calcified worms, tissue eosinophilia (4/4 cases), histiocytes, granulomas and foreign body giant cells (3/7 cases each) were the most consistent features, pointing to the fact that in the presence of such features an effort should be made to locate an etiology by taking more tissue sections.

Human subcutaneous dirofilariasis is a rare helminthic infection caused by filarial worms of the genus *Dirofilaria*, which is the natural parasites of dogs, cats, foxes, and wild mammals. *Dirofilaria* species belongs to the filarial nematodes, causes zoonotic infections in man, occasionally. Subcutaneous dirofilariasis is caused mainly by *Dirofilaria repens*, which causes subcutaneous nodules in and around the eye<sup>15</sup>. Human dirofilariasis due to *D. repens* has increasingly been recognized in India with most cases presenting with ocular manifestations<sup>15</sup>. *Dirofilaria* usually presents as subcutaneous nodule, either tender or nontender; occasionally migratory; and may be associated with an abscess. It is commonly found on the eyelids, scrotum, breasts, arms, and legs<sup>16</sup>. Mode of transmission of dirofilaria is through bite of an infected mosquito of the *Culex* and *Anopheles* species. Histologic

examination for species identification can be made by analysis of the length and morphology of the parasite; patients do not typically exhibit eosinophilia<sup>16</sup>. *Dirofilaria* is characterized by a relatively large size, thick cuticle, and prominent musculature with muscle cells extending far into body cavity<sup>17</sup>. Different *Dirofilaria* species can be distinguished by their size, thickness of cuticle, and presence or absence of longitudinal ridges<sup>17</sup>. The presence of thick laminated cuticles, large muscle cells, and wide lateral chords is diagnostic for this parasite. Histopathological sections usually show cross section of the parasite with surrounding granulomatous tissue reaction with an intense inflammatory cell infiltrate composed of neutrophils, lymphocytes, and plasma cells along with foreign body giant cells<sup>18</sup>. Despite *Dirofilaria* being a filarial nematode, the present study describes the cases separate from those of filariasis as it is an emerging zoonotic parasitic infection and not very well documented in the literature. The present study describes three cases of subcutaneous dirofilariasis affecting the eyelids. The most consistent tissue reactions were granulomas (3/3 cases), histiocytic infiltration, giant cells, and acute inflammatory infiltrate (2/3 cases each). Scanty tissue eosinophils were seen in one case.

Human cysticercosis is a potentially deadly infestation and is the consequence of ingestion of eggs of *Taenia solium*. Cysticercosis is the most common parasitic infestation of the central nervous system, muscle and subcutaneous tissue. About 54% of the patients present with subcutaneous nodules<sup>19</sup>. Clinical features of cysticercosis shows numerous small papules and nodules, cysts in subcutaneous tissue, skeletal muscles, or mucous membranes, urticaria from leaking cyst fluid<sup>19</sup>. Histological examination usually shows preserved parasitic morphology with calcospherules. The host tissue reaction ranges from epitheloid to histiocytic to lymphocytic proliferation with or without a capsule. The parasite appears in the well formed cyst as usually distorted and often mummified. But the hooklets were relatively preserved up to the late stage<sup>20</sup>. The present study describes tissue cysticercosis in chest wall in 2 patients and 1 each of abdominal wall and right thigh lesion. The histological findings include inflammation with eosinophilia(2/4 cases),

histiocytic granulomas with giant cells(2/4 cases), necrosis(1/4 cases) and discernible worms in the microscopic examination(3/4 cases).

Hydatid cyst disease is an endemic parasitic infestation caused by *Echinococcus granulosus* and it is an important public health problem in the Mediterranean countries, Middle East, Africa, South America, Asia and Australia<sup>21,22</sup>. It most commonly affects the liver (60–70%) while lungs are the second most common site (5–27%)<sup>21</sup>. If parasite passes liver and lungs, it may locate in any organ. Primary subcutaneous hydatid disease means that there is not any primary focus of hydatidosis. The main symptom of subcutaneous hydatidosis was mobile painless, slow growing mass and only 30% of the patients complained with pain. Kayaalp C *et al*<sup>22</sup> reported the incidence of subcutaneous hydatid disease as 1.5% among all cases of hydatid disease in endemic areas. Serology is a useful tool particularly for the differential diagnosis of hydatid liver cysts, however, it is usually negative (79%) for subcutaneous hydatid cysts. Microscopically, hydatid cyst has 3 layers. Innermost (germinal layer) is 10 - 25 microns, contains nuclei, gives rise to brood capsules attached by short stalk in infectious (fertile) cysts, often with daughter cysts. Also protoscolices (attached or separated) with double row of refractile, birefringent, acid fast hooklets 22 - 40 microns and 4 round suckers that comprise “hydatid sand”. Daughter cysts may merge and provide internal septation. The second layer is a laminated membrane beneath germinal layer, which is 1 mm thick, avascular, eosinophilic, refractile and chitinous; strongly positive for Periodic acid Schiff, and Gomori’s methanamine silver. Outer layer is dense fibrovascular tissue with chronic inflammatory cells, variable calcification develops after over a long period of time if the disease goes unnoticed<sup>23</sup>. The present study described a case of subcutaneous hydatidosis involving the right calf muscles, the tissue reaction showed a pericytic histiocytic infiltrate along with chronic inflammation, the debris on processing showed laminated membranes, germinal layer and occasional protoscolices.

Fine Needle aspiration cytology (FNAC) is a safe, reliable, rapid and cheap method of evaluating swellings. It is well tolerated by patients and usually done as an outpatient procedure.

In cases of suspected malignancy, FNAC is a good choice as it prevents spread and in cystic swellings can be therapeutic<sup>24</sup>. FNAC is currently being used for diagnosis of parasitic infections<sup>24</sup>. Utility of FNAC in parasitic infections such as cysticercosis, filariasis and hydatid cyst disease has been well documented, but is not without pitfalls<sup>25</sup>. The cytological specimen is then evaluated and smears are made of the aspirate for further evaluation. Parasitic infections are usually confirmed when there are demonstrable organisms in the smear along with other features of infections. Lymphocytic infiltration, particularly eosinophilic, along with giant cells, fibrous tissue are usually made out in the stained smears.<sup>26</sup> In cases where there are no discernible organisms or parts thereof, biopsy with histopathological examination may reveal the diagnosis. Histopathological analysis helps in understanding the interactions between the host and the parasite and aids in the diagnosis where cytopathology does not reveal a conclusive diagnosis<sup>27</sup>. In some cases like hydatid cyst disease, it is better to perform an excision than risk spillage of the hydatid fluid which can cause reactions<sup>9</sup>.

Parasitic infections are on an increase in the recent years. Many factors or combinations of factors contributing to disease emergence include ecological changes, such as those due to human activities or to anomalies in climate; travel and immigration, technology and industry; microbial adaptation and change and breakdown of public health measures. The emerging infectious diseases are also attributed to the population growth, ageing population, poverty and malnutrition, environmental pollution, deforestation, crowding, inadequate infrastructure, poor sanitation and water supply, global warming, development of antimicrobial/insecticide resistance etc. In addition to this the immunocompromised patients, including patients with AIDS, solid organ transplant recipients, and patients on immunosuppressive therapy for disorders, are at high risk for opportunistic parasites<sup>27</sup>.

### CONCLUSION

Histopathological features like granulomatous inflammation and tissue eosinophilia are strong indicators of a parasitic aetiology. Subcutaneous and intramuscular

filariasis, cysticercosis and hydatid cyst are well documented in literature whereas *Dirofilariasis* is an emerging zoonotic infection in the Indian subcontinent. Imaging techniques and fine needle aspiration can point towards the diagnosis with precision; however in the absence of characteristic features, histopathology can be relied upon to diagnose a helminthic aetiology.

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### Conflict of Interest

There has been no conflict of interest in the present study.

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