Original Article

A retrospective analysis of parasitic diseases diagnosed by tissue biopsy: a 7- year study

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Abstract

Introduction: There are wide varieties of parasites that can infect humans involving various tissues and organs. Parasitic diseases are common in developing countries associated with poverty and poor sanitation. Non-endemic developed areas also show increasing trend of disease as a result of immigration. Morphological identification of parasitic structure along with inflammatory reaction in the surrounding tissue forms the mainstay of diagnosis of parasitic diseases on histopathology.

Methods: The present study was a retrospective study carried out over a period of 7 years from 2010–2016 in the department of Pathology of TUTH, Kathmandu.

Results: 121cases (0.25%) of tissue parasites out of the total 47,220 biopsy specimen submitted for histopathological examination during the seven year period was observed. There was increasing rate of parasitic infection from 0.19 to 0.34% during 7- year period. Eight different types of parasites were detected. Hydatid cyst was the commonest (57 %) followed by Cysticercosis (35 %), Giardiasis (2%), Amoebiasis(2%) and one each of Myiasis, Fascioliasis, Tungiasis and Trichuriasis. The host tissue response to the cysticercosis and hydatid cyst showed fibrosis with inflammatory cell infiltrations composed of eosinophils, palisaded histiocytes, multinucleated giant cells. None of the cases showed well formed granulomas. All three cases of Giardiasis showed normal villi to crypt ratio with only increase in intraepithelial lymphocytes. Colonic mucosa in tichuriasis and amoebiasis showed increased eosinophils in lamina propia. Adjacent tissue in myiasis showed necrosis. Liver tissue in fascioliasis showed multiple well-formed granulomas and dense sheets of eosinophils and mononuclear cells.

Conclusion: Parasitic infections are common around the world. Histopathological analysis plays an important role in diagnosis of tissue parasitic disease. Histopathological features in response to parasitic invasion are important for differential diagnosis and often confirms the presence of parasitic infestation. The commonest parasitic infections were hydatid disease and cysticercosis. So these should be kept in the differential diagnosis of cystic lesion in both endemic and non-endemic areas. Improvement in sanitation and personal hygiene helpful in preventation of parasitic infestation.

Keywords: Tissue Parasites, Helminthes, Protozoa,

Introduction

Parasitic disease caused by helminthes and protozoan parasites affecting different organs is still a significant but neglected public health problem in the developing countries, in the poor and marginalized communities.[1] Serological assays are available for the diagnosis of parasitic infections but none of these are sensitive and specific to establish the diagnosis, cross react with other parasitic infections. These assays have decreased sensitivity when the lesions are calcified.[2,3]

So histopathological examination is a reliable way to diagnose parasitic diseases by detecting characteristic morphology of parasites. Failure to demonstrate the parasite does not exclude the possibility of infection. Inflammatory reaction in the surrounding tissue could give a clue about parasitic infestations. This study was aimed at determining the role of histopathology in diagnosis of parasitic disease and to assess histopathological changes in response to parasitic invasion of tissue.

Methods

This was a retrospective study of 121 cases of tissue parasites conducted at the Department of Pathology, TUTH over a period of 7 -years from 2010 to 2016. Parasitic diseases diagnosed in surgical biopsies were reviewed and analyzed. Demonstration of parasitic structures in tissues was diagnosed as parasitic disease. The data were retrieved from computer database. Data were entered in Excel spread sheet and analysis was done.

Results

During the study period a total of 47,220 surgical biopsies were received for histopathological examination. Out of which, 121(0.25%) cases of parasitic diaseases were diagnosed. There were 69 cases(57%) of Hydatid cysts followed by 43(35%) cases of Cysticercosis, 3 cases of Giardiasis, 2 cases of Amoebiasis and one each of Myiasis, Tungiasis, Trichuriasis and Fascioliasis (Table1).

The most common age group affected in Hydatid cyst was 21 to 30 years, male to female ratio of 1:1.3 with slight female preponderance. The youngest patient was 5 years old and oldest patient was 98 years old. Liver was most common site for Hydatid cyst 46% followed by lungs 39%, brain 6%, spleen 3%. One case each was seen in kidney, knee joint, muscle, and pancreas (Table 3).

Age distribution of Cysticercosis varied between 3 to 70 years. Majority of the patient were also between the age group of 21-30 years. Youngest patient was 3 years and the oldest patient was 70 years. Among total 43 cases, 26 were male and 17 were female with male to female ratio 1.5:1 with male preponderance. Among 43 cases of Cysticercosis, most common involved site was the subcutaneous tissue seen in 18 cases (42%) followed by skeletal muscle 13 (30%) cases, eye 8(19%) and brain 4(9%) cases (Fig1).

Myiasis was seen in 35 years old female in the breast. Trichuris trichuria was detected in caecum. Tungiasis seen in shaft of penis, pear shaped trophozoit of Giardia seen in duodenum, amoeba in colonic mucosa, part of ova of fasciola hepatica seen in liver.

Table 1. Annual Distribution of parasitic diseases at TUTH(2010- 2016)

Year	Hydatid cyst	Cysticercosis	Giardiasis	Amoebiasis	Trichuriasis	Tungiasis	Myiasis	Fascioliasis	Total(%)
2010	5	4	1	1					11(0.19)
2011	5	11		1					17(0.29)
2012	7	8							15(0.24)
2013	8	6							14(0.20)
2014	7	9							16(0.21)
2015	14	3	1			1			19(0.26)
2016	23	2	1		1		1	1	29(0.34)
Total	69	43	3	2	1	1	1	1	121(0.25)

Table 2. Frequency of Parasitic diseases

Parasites	Frequency (n)	Percentage (%)
Hydatid cyst	69	57
Cysticercosis	43	35
Giardiasis	3	2
Amoebiasis	2	2
Myiasis	1	1
Tungiasis	1	1
Fascioliasis	1	1
Trichuriasis	1	1
Total	**121**	**100**

Table 3. Sitewise distribution of hydatid cyst

Site	Frequency(n=69)	Percentage (%)
Liver	32	46
Lung	27	39
Brain	4	6
Spleen	2	3
Knee	1	2
Kidney	1	2
Muscle	1	1
Pancrease	1	1
Total	**69**	**100**

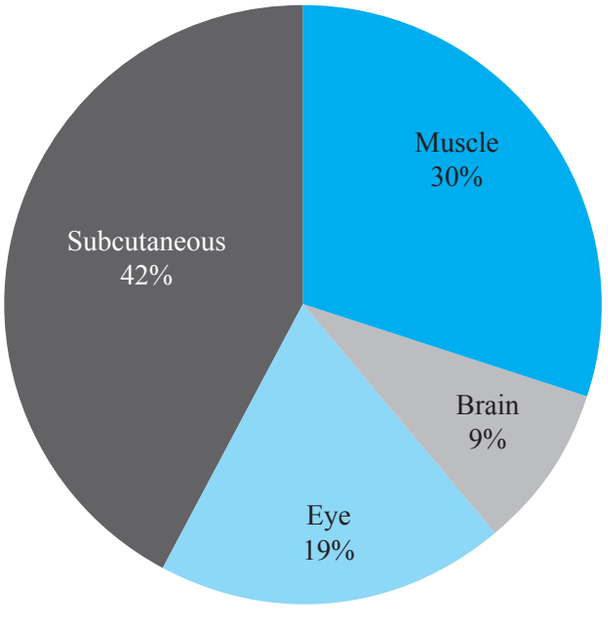

Figure 1: Sitewise distribution of Cysticercosis

Discussion

Parasitic infections are more common in rural or developing areas than in developed areas. Infections are associated with poor sanitation and unhygienic practices. Extent of the disease depends on burden of parasites, types of tissue response and host immunity. People with weak immune system can suffer from severe and fatal disease.[3]

During the study period of 7 years, 121(0.25%) cases of parasitic diseases were diagnosed in surgical biopsies (Table1). Similarly Kim J et al and Chi JG et al found 0.18 % and 0.33% parasitic disease during period of 10 years and 20 years respectively. [4,5]Present study showed increasing rate of parasitic infection 0.19 to 0.34% during 7 year period (Table1). In contrast, due to remarkable economic growth, improvement of hygiene and better diagnosis and treatments, parasite infection rates have been remarkably decreasing in Korea.[6]

In the present study, among helminthic infections, Hydatid cyst and Cysticercosis constituted majority of tissue parasitosis. Out of 121 cases, Hydatid disease was most common parasitic disease constituting 69 (57%) cases followed by cysticercosis accounting for 43 cases (35%). Choi et al analyzed 150 cases of parasitic diseases. In their study, cysticercosis was the most common parasite.[6] Kim J et al and Chi JG et al in their study also found cysticercosis as most common parasitic disease.[4,5]

Hydatid disease is a zoonotic infection caused by larval stages of cestode species of the genus Echinococcus. E. granulosus and E. multilocularis cause cystic echinococcosis (CE) and alveolar echinococcosis (AE) respectively. The two other species, E. vogeli and E. oligarthrus, cause polycystic echinococcosis but rarely been associated with human infection.The infection is endemic in especially in sheep breeding areas. Dogs are definite host. Sheep and goats are the intermediate host. Man is infected after consuming contaminated food with dog feces containing egg.[7]The most common localization for hydatid cysts is liver followed by lungs. But any organ of the body can be involved, but that is rare. It most commonly affects liver (50% -77%) followed by lungs (15-47%), spleen (0.5-8%), kidney (2-4%), musculoskeletal system (1-4%), pancreas (0.14-2%), brain (1-2%). [8-12]

In our study, liver was most common site of involvement seen in 46% followed by lung 39%, brain 6%, and spleen

3%. One case each of hydatid cyst was seen in muscle, pancreas, kidney and knee. Various studies also showed higher incidence of liver involvement than lung,[8,13,14]which is similar to our study. In contrast, a study done by Parija et al found lung as the commonest site followed by liver.[15] Present study showed 10 cases (14.4%) of hydatid cyst in unusual locations. Sarkar S et al also found 6% in unusual locations.[14]Various other authors have reported hydatid disease in unusual sites in muscle, knee, pancreas, brain. [9,11,12,14,16]The preoperative diagnosis for hydatid cyst in atypical localization is difficult, mimicking tumor. Diagnostic suspicion must exist especially in patients coming from endemic areas .[16]

The age of the patients ranged from 3-78 years with slight female predilection (1:1.3). The most common age group was 21-30 years in present study. Kayal A and Rao SS et al also showed female predilection with mean age group of 40 years. [8,13]In contrast Shrikanth et al and DK Lado et al reported highest distribution in male. [17,18]

Cystic ecchinococcosis occurs in age groups from younger than 1 to over 75 years. In endemic area, most hospital cases have recorded age groups between 21 and 40 years.[7]

Histopathology showed cyst wall comprising of inner germinative layer and acellular eosinophilic laminated structure of cyst wall. Protoscoleces with portion of hooklets seen in 10 cases. Outer host layer showed fibrosis with infiltrations of histiocytes, chronic inflammatory cells composed of lymphocytes, plasma cells and eosinophils.(Fig 2)

Cysticercosis was second common parasitic disease in the present study. Cysticercosis, a helminthic infection caused by larval stage of pork tape worm, Tenia Solium, currently remains one of the most common human parasitic infestations worldwide. Human beings acquire cysticercosis through ingestion of T-Solium eggs in contaminated and undercooked food. Thus, vegetarians and other people who do not eat pork can also acquire cysticercosis .[19]

Among 43 cases of cysticercosis, majority of the cases (42%) were seen in subcutaneous tissue followed by the skeletal muscle (30%) (Fig1). Similarly, various other authors also found that subcutaneous tissue as a most common site .[4,6] In contrast Chi HS et al study showed that skeletal muscle was most common site comprising 37.2 % followed by subcutaneous tissue 32.4%.[20] Present study showed eye involvement in 19% of cases followed by CNS only in 4 cases (9 %) in contrast to study done by Choi WH et al study which showed CNS involvement in 43.5%. [6]

Male predominance was seen with male to female ratio of 1.5:1 in age group of 21-30 years. Similarly, study done by Choi WH et al also showed male predominance**.** [6] In contrast, Kim J et al study showed that females were affected more than males.[4]

Histopathology revealed a thin lightly staining bladder wall. Bladder wall consists of of tegument with wart like protuberance. Beneath the teguments are small rounded pyknotic nuclei surrounded by fibrillary matrix. Parenchymatous portion showed folded spiral canal and scolex with two sucker and rostellum with hooklets seen in 10 cases only. Surrounding host response composed of lymphocytes with eosinophils, palisades of histiocytes and fibrosis.(Fig 3)

Parasitic protozoa are the most common pathogenic gastrointestinal parasites worldwide affecting millions of people in developing countries.

Two cases of entaamoeba histolitica were diagnosed in the present study. Histopathology showed round trophozoite with ingested RBC in the cytoplasm. Entaomoeba histolytica is a protozoan parasite. It is transmissible by fecal –oral route primarily infects human bowel. It exists in two forms, trophozoite that can invade multiple organ system and cyst form that can colonize. It can cause bleeding, diarrhea to asymptomatic colonization. History, stool studies and biopsy play an important role in differentiating E histolytica associated coltis from infammatory bowel disease and invasive bacterial dysentery.[21]

The most reliable and dependable diagnostic technique for amoebiasis is the demonstration of the trophozoites in specimens obtained from patient. Trophozoites may not be found in biopsy materials because they disintegrate and disappear. A number of serological tests have been found useful in confirming the diagnosis of invasive amoebiasis but limitation in routine use.[22]

In the present study, Giardiasis was seen only in 3 cases(2%). Trophozoites were found in duodenal mucosa. Giardiasis is caused by Giardia lamblia, which is a pear shaped flagellated protozoa spread by ingestion of contaminated food and water with infectious cyst, trophozoites emerge from the cyst in the duodenum and attached to the small intestinal mucosa. It cause asymtomatic carrier to a severe malabsorption syndrome. Giardiasis is confirmed by demonstration of trophozoites in duodenum adhering

to intestinal epithelium and in faeces.[23]All three cases showed normal villi to crypt ratio with only increase in intraepithelial lymphocytes. Oberhuber et al analysed histological changes in 80 cases of giardiasis, found mild villous flattening in 41.2%. Higher grades of villous flattening were not observed. In their study, intraepithelial lymphocytes were not significantly increased. Giardiasis is not associated with specific histological changes, it ranges from minimal to severe enteroyte damage, villus atrophy and crypt hyperplasia. Therefore, Parasites should be searched carefully for diagnosis .[24]

Present study showed adult worm of trichuris trichuria in the colon. Long slender worm with wall consisting of the thick outer cuticle and barrel-shaped eggs was seen in the lumen.(Fig 4) Adjacent colonic mucosa showed mild eosinophilic infiltrations in the lamina propia. Khuroo M et al also found only slight increase in lymphocytes, eosinophils and plasma cells in lamina propia at places with absence of cryptitis and crypt abscess [25]Trichuristrichuria is intestinal helminth of class nematode inhabiting the human caecum. It is endemic in tropical and subtropical countries with poor sanitation, malnutrition caused by ingesting embryonated eggs. [25] Patient is symtomatic only in heavily infected. It causes colitis which may be fatal in severe case. Diagnosis of parasite infections made by identifying egg in stool specimen may be difficult in cases of infection only with a few male parasites. [26]

One case of tungiasis was seen in penile shaft of 65 years old patient. Histopathology showed parts of an insect including thick outer cuticle, ovum. (Fig 5) Tungiasis is an ectoparasite diasease caused by penetration of skin from gravid female flea Tunga penetrans(also known as chigoe flea, sand flea, chigo) preferring warm, dry climate, sandy soil and dust endemic to Central and south America, sub-Saharan Africa, the Caribbean. Common sites are feet and legs, especially the toes and subungual and periungual region. [27]Junior MML et al also report a case of tungiasis in penis in 7 year old patient. Although a rare entity in the genital areas, it should be considered in the differential diagnosis of other genital lesions.[28]

A single case of myiasis was found in 36year old female breast. Histopathology showed extensive areas of necrosis with multiple larvae of Diptera. Human myiasis is infestion by fly larvae (maggots) of Diptera (flies with two wings). It was distributed worldwide with common occurrence in poor socioeconomic region of tropical and subtropical countries. Myiasis was first described by HOPE in 1840. Since then many cases of myiasis affecting different organ have been described. It usually affects skin wounds, intestine and host orifices.[29] Most common is human botfly (DermatobiaHominis), endemic in Central and South America. Cordylobia anthropophaga (tumbu fly) is endemic in West African subregion. Khan DG also reported myiasis presenting as a long standing breast mass and mimicking a neoplasm.[30] Adisa CA reported Fruncular myiasis of the breast caused by the Tumbu fly. Cutaneous myiasis of the breast is very rare, only few cases have been reported, mainly due to human botfly.[29]

Hiatological features of parasites showed outer thick cuticle that was covered with pigmented spines. Striated muscle was seen under the cuticle.

A single case of hepatic mass caused by Fasciola hepatica seen in 66 year old female clinically mimickingof malignancy. Similarly Yilmaz B et al also reported a case of fascioliasis clinically suspected of malignancy.[31] Fasciola hepatica is a zoonotic liver fluke of the class Trematoda that infects the liver causes fascioliasis which is common in developing countries. Definitive hosts are man, sheep, cattle and goat. Humans become accidental hosts infected by eating water plants. Identification of fasciola hepatica eggs in the stool is diagnostic of fascioliasis. The parasites do not pass eggs in the acute stage of the disease before they become adults. Serologic tests are quite sensitive, but they may cross-react with other parasitic infections, such as echinococcus.[31]

Histopathological examination of liver biopsy revealed parasite part of egg with yellow brown wall within multinucleated giant cell. Adjacent areas showed multiple well formed epithelioid granulomas and dense sheets of eosinophils and lymphocytes, plasma cells. (Fig 6)

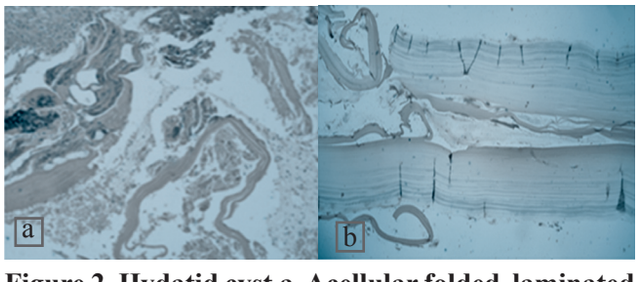

Figure 2. Hydatid cyst.a. Acellular folded laminated membrane surrounded by multinucleated giant cells and host fibrous tissue (left) (H&E, 40X). b. Acellular laminated membrane(right) (H&E 400X)

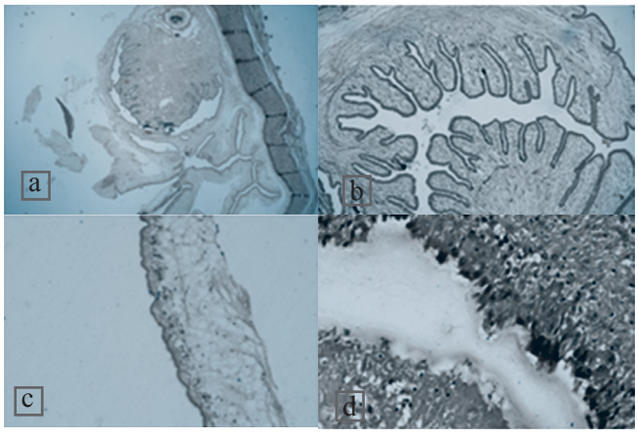

Figure 3. Cysticercus: a. Bladder wall with parenchymatous portion of folded spiral canal and fibrous host tissue (left) (H &E 40X). b. Folded spiral canal (right)(H&E400X). c. Three layers of cysticercus, loose stroma (star), tegumental cells (closed arrow), wavy tegument (open arrow (Left)(H&E400X). d. Fibrous wall with palisaded histiocytes. (arrow head) (right) (H&E 400x)

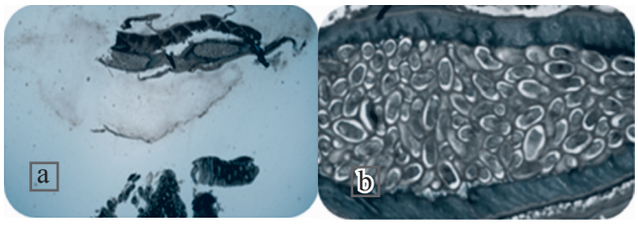

Fig 4. Trichuris Trichuria. a. Long slender worm with adjacent colonic mucosa. (H&E, 40X), left. b. Barrel shaped eggs with outer cuticle (H&E 400X),right

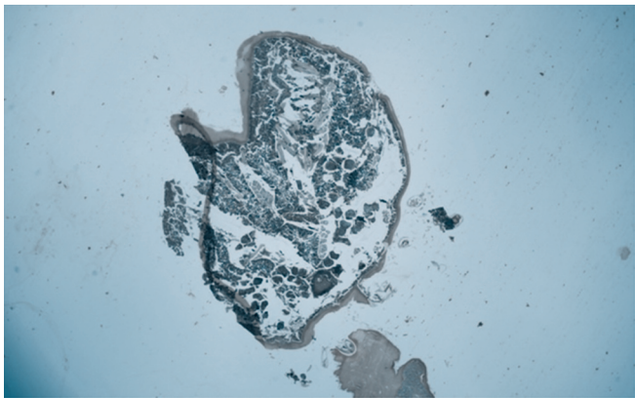

Figure 5. Tungiasis. Thick cuticle (open arrow) with external orifice of insect (arrow head). Internal organs within cavity including ovary containing eggs (star) , tracheal ring (closed arrow)(H&E,40X)

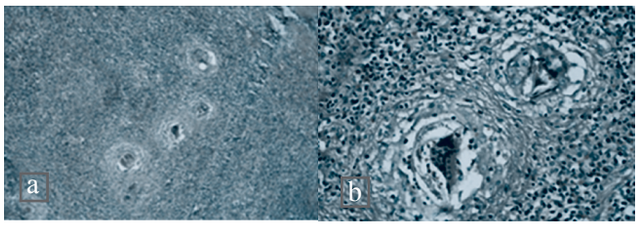

Figure 6. Fascioliasis. a. Multiple well formed granulomas with sheets of eosinophils. (left) (H&E 40X). b. Part of egg with brown wall within multinucleated giant cells .(right) (H&E, 400X)

Conclusion

Hydatid cyst and cysticercosis are the commonest parasitic lesions. Cyst in unusual location present difficulties in clinical diagnosis. Histopathological examination of the lesion can make the confirmative diagnosis based on morphological identification and surrounding host tissue reaction. The host tissue response is variable ranging from mild to moderate chronic inflammatory cell infiltrations composed of predominantly eosinophils, few lymphocytes, plasma cells, histiocytes. Therefore, eosinophilia may indicate the presence of parasites. Careful search for the parasites should be made in biopsy in minor pathological changes in adjacent tissue. Maintaining good sanitation and personal hygiene can reduce parasitic infestations.

Conflict of Interest: None declared

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