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Peri Cholecystectomy Bile Analysis for *Fasciola hepatica* Eggs in Sulaimani Teaching Hospital

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Abstract

Fascioliasis is one of the oldest zoonotic infestations, which returned back to 3000 years B.C., it is caused by Fasciola hepatica or gigantica. It is a widespread disease throughout the world; nearly (2.4 – 17) millions of people is infected. Human is an accidental final host by ingestion of contaminated plants and water by metacircaria. Most of patients suffer from right upper abdominal pain with eosinophilia. Diagnosis is made on clinical background with imaging studies or laboratory (like stool and examinations biliary aspirate, intradermal test or ELISA to detect antibody). It must be taken into consideration as newly emerging infestation in our country.

The aim of this study is to highlight on the magnitude of Peri Cholecystectomy Bile Analysis for Fasciola hepatica in Sulaimani province, Kurdistan region and even in Iraq as a whole. A prospective study was performed at Sulaimani Teaching Hospital. Bile samples were collected from who had cholecystectomy. Microscopical analysis was archived for finding egg of the parasite.

In 129 samples, there were 7 (5.4%) positive cases of Fasciola hepatica 2 were adult worms, all were swimming in the gall bladder. 5 (3.9%) out of 7 (5.4%) samples were females and only 2 (1.5%) of cases were males. 4 (3.1%) out of 7 were from Sharazur terrain, and 3 (2.3%) of them from the center of

Sulaimani city.

Fascioliasis is a zoonotic problem. It must be put in differential diagnosis of any vague upper abdominal pain. It is a public health issue, so health worker must give awareness to the society about this disease, which is more easily preventable.

Keywords: Fasciola hepatica, bile analysis in Fascioliasis, liver fluke, diagnosis of liver flukes

1. INTRODUCTION

Fascioliasis is one of the oldest parasitic infestations of human being that discovered arround 3000 years B.C [1]. The eggs of the Fasciola hepatica found in soil samples from the pelvis of human skeletons in Germany in 2003 [1,2,3]. It is a zoonotic disease caused by a parasite named Fasciola hepatica or Fasciola gigantica, in the Phylum of Plathelminthes, Class of Trematoda, Genus of Fasciola and species of F. hepatica or F. gigantica [4,5].

Human Fascioliasis is recognized by WHO as one of the "neglected tropical diseases" [6]. In Sulaimani first case reported in 1992 by accidental finding of the adult worm in the biliary duct after exploration of common bile duct (CBD) by Hawrami et al [7]. The first successful Endoscopic Retrograde Cholangiopancreatogram (ERCP) management of this disease by Ezzat et al in 2010 [8]. After that a case report of adult Fasciola hepatica that was swimming inside the gall bladder after cholecystectomy was found in 2010 [9]. Also Fascioliasis is reported as an endemic disease in our neighbor countries like Iran in 2015 [10] and Turkey [11].

Epidemiology: In the past Fascioliasis was limited to a specific geographical area, but nowadays it is a widespread disease throughout the world [12]. Reported cases of infected humans account nearly for (2.4 – 17) millions [13], more in Egypt and Peru [14]. The lacks of familiarity by clinicians in non-endemic areas have led to delay in diagnosis and increase rate of complications [15]. Currently the Fascioliasis is emerging in Iraq and it is often a neglected disease [16]. So it is not uncommon to see cases of Fascioliasis in non-endemic areas. The cause is importation of contaminated vegetable to higher consuming markets of non-endemic large cities [12]. This may be pivotal explanation of the wide geographical distribution of disease [17].

Morphology: The features of the Trematoda class are the same. They have a digestive system, protonephridial excretory system, orthogonal nervous system and a mixed reproductive system (hermaphrodite) [4]. The adult of F. hepatica measure about (25-30 mm) by (10-15mm), while F. gigantica is measured up to (75 mm). They are large, flat, brown colored (greenish when they are in bile) and leaf like shape; their anterior part is broad and covered with scale-like spines for catching the infected part of the host [12]. The eggs are oval in shape, yellowish brown in color and the size is about (130-150 µm) by (60-90 µm) [4,12].

Life cycle: It starts when the eggs of the parasite are deposited in tape water (22-26˚C) from the stool of infected human being or animals like (cattle, sheep, pigs, buffaloes and donkeys) [12,16]. The miracidia appear, develop, and hatch in (9-14) days [18]. Then they invade lymnaea specie of freshwater snails (intermediate host), in which they multiply as sporozoites and redia for (4-7) weeks [19]. They leave as free-swimming cercaria that subsequently attach to watercress, lettuce, alfalfa, mint, parsley and khat([12,16,18,19]. They remain suspended in water and encyst over a few hours. Human being (occasional final host) is infected after consuming the contaminated vegetable, washing plates or drinking contaminated water [12,20]. Inside the duodenum the larvae exist in the first week of ingestion and migrate through the bowel wall and peritoneal cavity (after 4 weeks). The juvenile larvae penetrate the liver through Glisson capsule after 48 hours (this is acute larval, hepatic or invasive stage of human infection), and enter the liver. Then migrate throughout hepatic parenchyma till reach the biliary system, where they become adult worms within (3-4) months from initial infection

till laying eggs [4,12,16,20].
Mature flukes consume hepatocytes, duct epithelium and reside for years in the hepatic and biliary tree and sometime in the gall bladder (this is the chronic or biliary stage of disease) [12]. The adult worm can produce eggs in 4 months after infestation (range 3-18 months). Later on they go to the intestine through sphincter of Oddi and coming out with feces to continue the cycle again [4,12].
Clinical manifestation: It is in two phases, acute or chronic [6,10]. Signs and symptoms depend on the worm burden, duration and phase of infection [21].
In the acute phase, the immature parasite migrates through the liver parenchyma and digest hepatic tissue, which lasts 3-5 months [12,21)]. Acute Fascioliasis is simulates clinically to that of acute cholecystitis including right upper abdominal pain, fever, nausea and vomiting with a significant eosinophilia [22]. Also the patient may have hepatomegaly, ascites, subcapsular hematomas, hepatic necrosis, liver cyst or abscess, diarrhea and hemobilia with severe anemia [22,23,24]. Occasionally the patient may present with cough due to pulmonary infiltration [22]. Can be seen as extra hepatobiliary in other anatomical locations as subcutaneous tissue, pancreas, eye, brain and stomach wall [25].
The chronic phase; begins nearly 3-6 months after consumption of metacercariae, when the parasite enters the biliary tree [12]. It may stay asymptomatic for longer than 10 years [26]. Symptoms usually reflect biliary obstruction with colicky pain in right upper quadrant and epigastric region [25,27,28]. It may also cause inflammation, epithelial hyperplasia and fibrosis, which leads to biliary obstruction, cholangitis and pancreatitis [25,29], liver mass or abscess, anemia and weight loss [30]. In addition, hepatic cirrhosis have been reported in infected children [31] and adult, especially those with high-density infections [32,33]. It is also a cause of intrahepatic cholangiocarcinoma [34)].
Sometimes, acute and chronic phases can overlap; which commonly has seen in endemic areas [35].
Diagnosis: when Fascioliasis is suspected clinically, it can be confirmed through laboratory or imaging techniques.
Several laboratory techniques are available. They include direct parasitological examination of the stool and biliary aspiration for presence of eggs or rarely the parasite [11,36]. Intradermal test and stool antigen detection are also used as a diagnostic test in all stages of the disease. They also evaluate the process of the treatment [36]. Enzyme-linked immunoabsorbent assay (ELISA) detects antibodies against cathepsin L1. It is highly sensitive and specific in the acute phase of the disease, also called Fas2-ELISA [37,38].
Imaging techniques include:
Abdominal ultrasound (US): in acute phase, focal areas of increased echogenicity, multiple nodular or irregular lesions of variable echogenicity or a single, complex mass in the liver looking like malignancy can be detected [35,39]. In the chronic phase US is less specific, may show adult parasites in gall bladder or biliary tree [11,40,41].
Computed tomography (CT) scan: In acute phase; multiple hypodense lesion seen mimic hepatic metastasis with subcapsular location (the shape and positions are changed with time) [42]. Other findings are subcapsular hematomas, and cystic calcification [19,42,43]. After the first month of infestation, multiple hypodense nodules, tortuous, tunnel-like branching lesions are visualized in the subcapsular region [42]. In the chronic phase necrotic granules are seen as single non-contrast enhanced irregular hypodense masses in the liver parenchyma and they are more centrally location [44,45].
Magnetic Resonance Imaging (MRI): In a T1-weighted 3D image shows homogenous contrast enhancement, in T2-weighted display homogenous hyperintense areas [24,46,47].
Endoscopic Retrograde Cholangiopancreatogram (ERCP): is increasingly used as a diagnostic and therapeutic tool for human Fascioliasis especially in chronic phase [23].
The aim of the study is to detect the incidence of Fascioliasis in Sulaimani governorate and detect undiagnosed cases that presented as other causes of biliary problems. It is crucial to undertake thorough measures as any delay in diagnosis may lead to potentially morbid conseqeunces.

2. METHODS AND MATERIALS

A prospective study done in Sulaimani Teaching Hospital from March 2012 to March 2013. To conduct this study, ethical permission was approved by the ethical committee school of Medicine, faculty of medical sciences/university of Sulaimani, Kurdistan Region of Iraq. After consent been taken from patients, a collection of bile samples (not less than 1cc) after cholecystectomy either laparoscopic or open, elective or emergency. Each sample labeled and recorded according to questionnaire sheets. The samples kept in syringes, saved in refrigerator in 4˚C. The samples later transferred to college of veterinary /Sulaimani University to be examined under (Olympus double lens)microscope, searching for eggs of the liver flukes.

For any positive sample containing Fasciola eggs, the patient has been informed and brought back to hospital for complete clinical evaluation. The patient asked about remaining signs and symptoms postoperatively and similar clinical findings among other family members. Stool samples sent for parasitology lab for eggs of Fasciola. Complete blood picture for white blood count and eosinophilia, liver function tests [total serum bilirubin (T.S.B), Serum alanine transaminase (ALT), Serum aspartate transaminase (AST) and Serum. alkaline phosphatase (ALP)] and imagine studies.

Histopathological report has been reviewed for any gall bladder fibrosis and metaplasia. Finally all the infected patients were treated with Triclabendazole or benzimidazole.

Data analysis: IBM Statistical Package for the Social Science (SPSS) version 20.0.0 used.

3. RESULTS

After collection and analysis of 129 bile samples under microscope, seven (5.4%) were positive for eggs of liver flukes. From those two of the positive samples were also containing the adult worm; the latter is large enough to be seen by naked eye as shown in the table (1).

Table 1: shows values of positive samples for eggs of Fasciola in the bile sample.

	Egg found in bile	Note
Positive	7 5.4%	
Negative	122 94.6%	
Total no.	129	

Figure no. (1) shows the adult worm which is Fasciola hepatica and it is (27 mm) in length. Figure no. (2) shows scale-like spines of the adult worm. In the Figure no. (3) it is worthwhile to mention that the parasite which was actually yellowish-brown has been transformed to a greenish appearance due to coloration with bile. In figure no. (4) the eggs oval in shape non-embryonated with operculum seen under microscope.

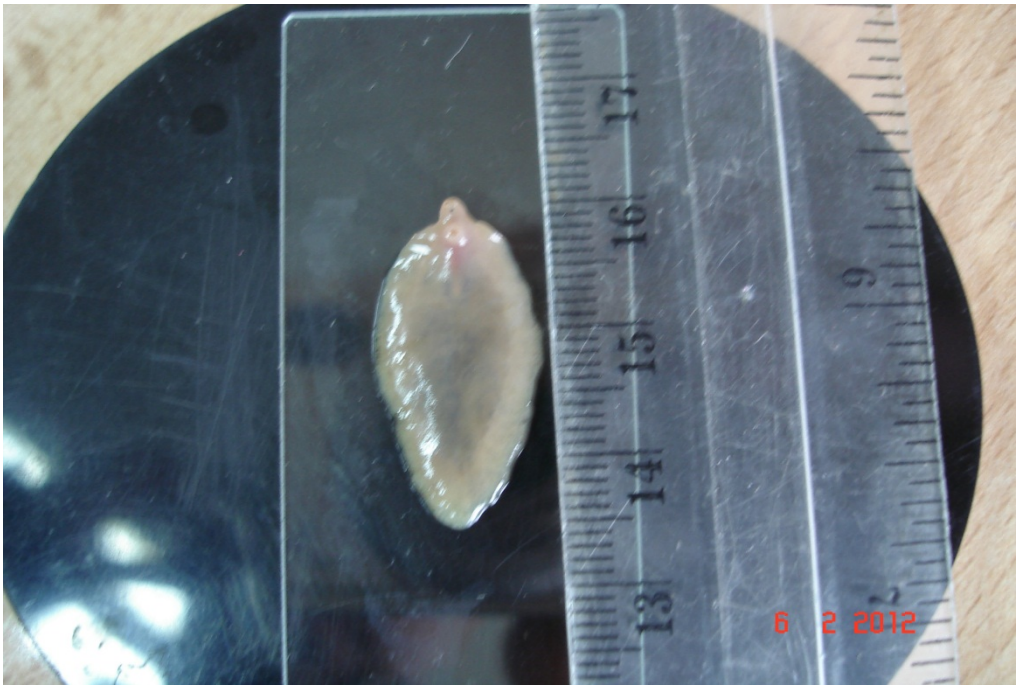

Figure 1: Shows adult worm with its length.

Figure 2: Shows the scale-like spines of Fasciola hepatica.

Figure 3: Shows adult worm over the gall bladder.

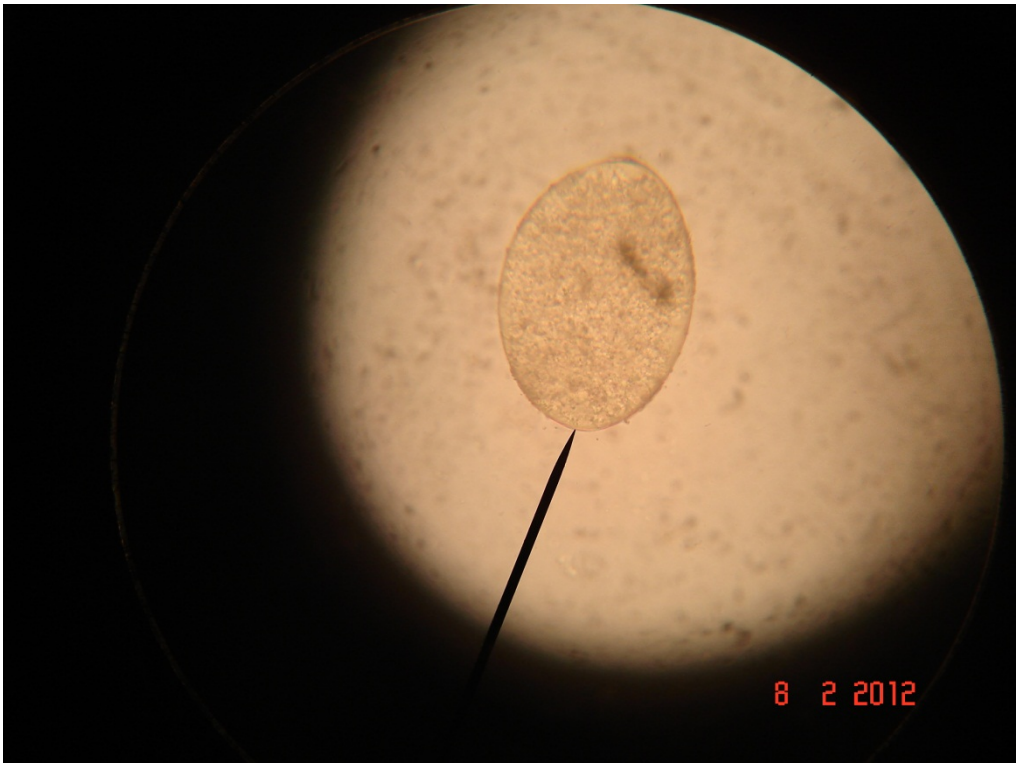

Figure 4: Shows the egg of parasite found in bile under microscope.

In this study 109 female patients underwent cholecystectomy, 5 (3.8%) of them were positive for liver flukes and only one case has shown adult worm (0.7%). Twenty of them were males with 2 (1.5%) positive result and only one adult worm, as shown in table no. (2).

Table 2: Shows gender relation with Fascioliasis.

	Female	Male	Note
Positive	5 3.8%	2 1.5%	
Negative	104	18	
Total	109	20	

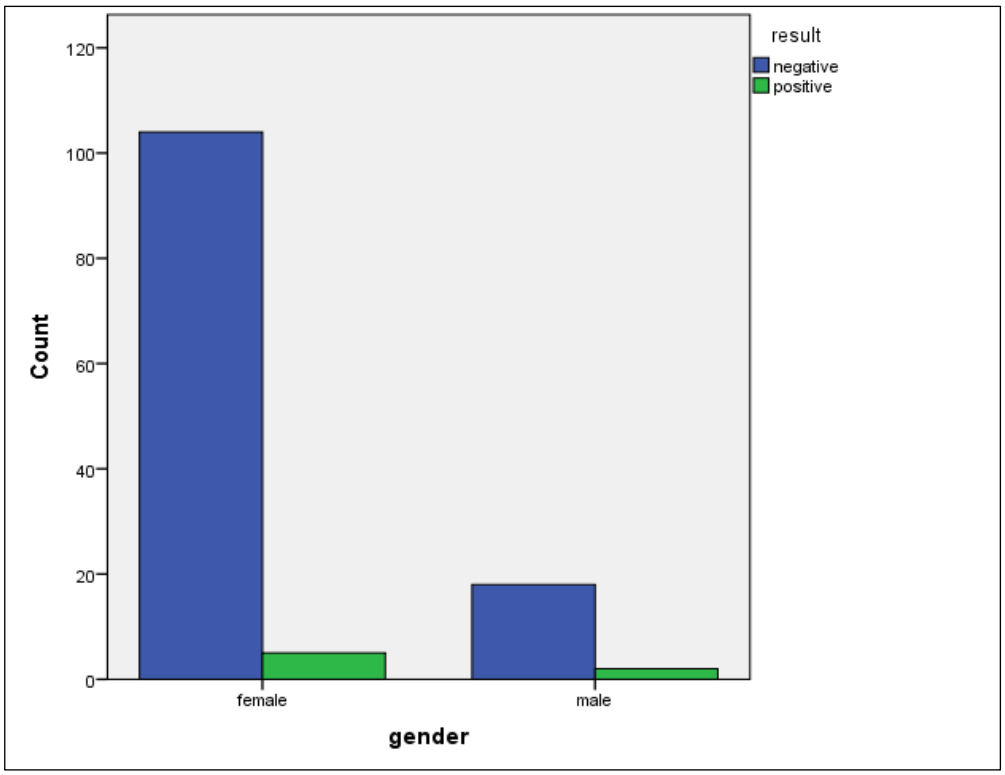

Figure 5: Shows relation between male and female with the disease.

One of the identifying point of this research is that Fascioliasis is more common in Sharazur terrain (Said Sadiq, Halabja Shahid and Halabja Taza) it is 4 out of 7 positive cases (57%), and 3 is from center of Sulaimani (43%), as shown in table no. (3).

Table 3: Shows geographical distribution of Fascioliasis in Sulaimani governorate.

Terrain	Positive	Negative	Total
Center of Sulaimani	3	88	91
Sharazur	4	17	21
Garmyan	0	14	14
Pishdar	0	3	3
Total	7	122	129

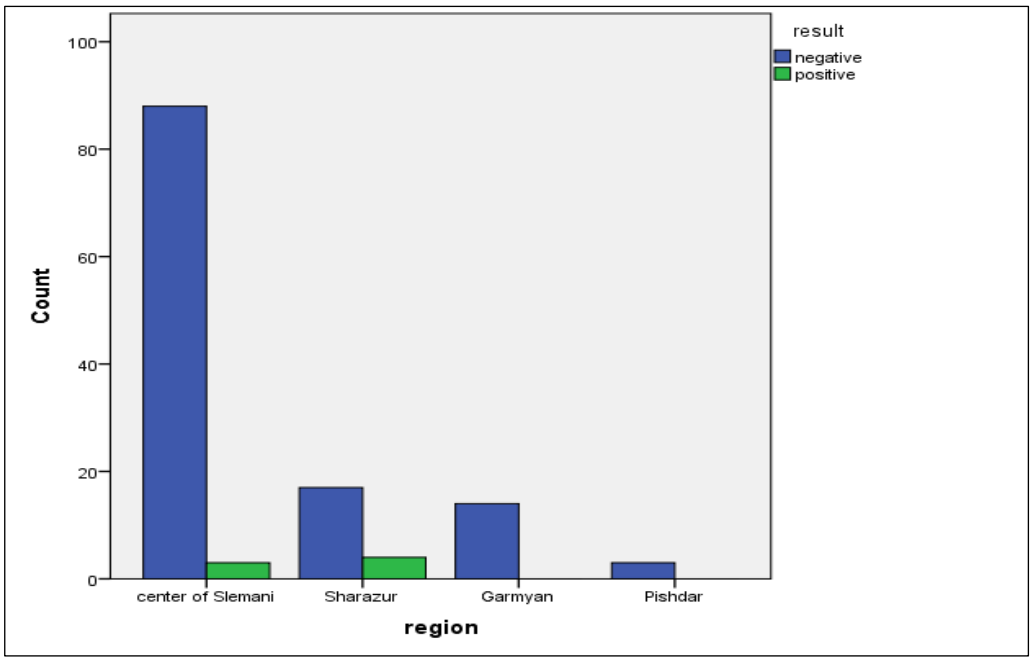

Figure 6: Shows geographical distribution of the disease.

4. DISCUSSION

The present study explains that the liver fluke become a health problem in Sulaimani province and this should be considered as suspected biological hazard, as our region is gradually becoming endemic to the parasite resembling some of our neighbor countries like Iran and Turkey [10,11,16]. It is believed that the dissemination of the infection to the new areas on non-endemic regions is due to the highly adaptable capacity of both the parasite and the lymnaeid snail hosts to challenging meteorologic condition [48]. So it must be take it in consideration in our country as a prevailing infestation. Fascioliasis should be considered in the differential diagnosis of patients presenting with vague abdominal pain when not reaching the diagnosis.

As shown in this study out of 129 bile samples 7 (5.4%) was positive, the positive results mean an egg has been found inside the bile samples. It is not worthy to mention that adult worms have been elicited inside the gall bladder of 2 patients during sampling. After establishing Kurdistan Centre for Gastroenterology and Hepatology in Sulaimani province and performing ERCP procedure, Hawrami et al were able to detect this parasite in 18 patients, which were present in different hepatobiliary procedures [16]. Later on they started searching for the source of this problem in our region. It has been discovered that the culprit is a snail (Lymnaea Sp.) which is found in the Sharazur and Penjwen by Hawrami et al [49], which is needed for completing the life cycle of the parasite.

Sharazur (included Said Sadiq, Saraisubhanaga, Halabja, Halabjay Taza and Khurmal) was behind the most positive samples. Four (3.1%) out of 7 were actually detected single handedly in this region. The possible explanation for this is believed to be due to 2 factors; firstly this area is well known for its aquatic plants called (water cress and mint). Secondly this large terrain contains a high number of snails and metacercaria as shown by Hawrami et al it was more in Sharazur (49).

The other 3 positive samples (2.3%) are belong to central of Sulaimani (the major city and the districts around it including Chwarta, Bazyan, Piramagrun and Arbat), this is may be due to the vegetables been transported from outside mostly from Sharazur.

There is a significant difference between female and male infection rate that includes 5 (3.8%) positive females from total 7 samples. There may be 2 possible factors; first most cholecystectomized patients were females in our study and it is accepted by Nakeeb et al research which states that around 88% of operative cases of gall bladder removal were female [50]. Second factor is the lack of proper sanitary education among housewives in regard to vegetable clean up like for instance wearing gloves and using specialized vegetable bleaches, this lead to transmission of the metacercaria attached to the hands of the lady unaware about them. This is synchronous with Hawrami et al that had more female cases which were 5:1 for females [16].

Both Fasciola hepatica and Fasciola gigantica can cause human infestation, but Fasciola gigantica is rare, it's reported to cause Fascioliasis as shown by Le Thanh Hoa et al [51]. In our study the causative organism is Fasciola hepatica as shown in figure (1). The characteristic feature of it, which is the length is (27 mm) and the width is (12 mm), the anterior aspect is the broader part. Also the scale-like spines are shown in the figure (2) which is magnified under microscope. The scale is used by the parasite for catch itself on the host, which is use by the parasite to function as a ventral sucker and they are connecting with parasite's digestive system.

This parasite is swimming inside gall bladder and can be seen during cholecystectomy. It is visible by naked eye over the surface of the organ after being incised, as shown in figure no. [3], which is again in line with that of Hawrami Tahir, in which as a matter of fact as he found a living parasite inside the gall bladder [9].

After the bile samples taken from the patients and underwent analysis under microscope, the eggs of Fasciola found in the 7 samples, as shown figure (4). The eggs have had characteristic feature of Fasciola, as it is oval in shape, bile color and it has an operculum, and the latter is specific for of the eggs of Fasciola species [4]. Another characteristic feature is non-emberyonated egg, which is found only in trematodes [52].

The analysis of the bile is crucial to detect this disease in undiagnosed cases, where they undergone cholecystectomy for biliary symptom which might be Fasciola rather than the gall stone. This is in agreement with the 3 case reports that done by Adnan Kabaaliogulu et al [11]. He found that 3 cases presented with right upper quadrant pain, without gall stone and no biliary symptoms. Mr. Kabaaliogulu methodically did the process of bile aspiration under ultrasound guidance, later on he sent the aspirate for parasitological examination and they found the egg of Fasciola in sample.

Whereas some persons remain undiagnosed, they are asymptomatic, at time it may progress to complication as serious as cirrhosis and carcinoma [34,53]. Delay of diagnosis is related to several resons: (i) the clinician is either not well aware about this disease because it is a rare condition even in endemic areas; (ii) the sign and symptoms similar to that of other hepatic and biliary disease; (iii) scarcity of serological tests in many countries specially in developing counties; (iv) the eggs of parasite seldom found in stool and more frequently detected in the bile [11]. So technically sending bile for parasitological investigation will be more eventful in elucidating the eggs, which is informative and less costly, this enhance the chance for early diagnosis and treatment.

When the Fasciola adult worm isolated during operation, the patient treated instantly with anti-helminthic medications. But for the remaining five samples where only eggs have been picked up, we maintained our contacts with such group in order to call them back and giving them proper treatment. The usual course of anti-helminthic medications is as follow which is either Triclabendazole or Benzimidazole. Triclobendazole was given as a single dose orally (10 mg/kg). But Benzimidazole is well-tolerated and highly effective against immature flukes [52, 54].

It's common to see eosinophilia after parasitic infestations [12], and among the seven cases five of them were actual cases of eosinophilia, and this result coincides with Hawrami et al [12,16]. In the rest of the cases eosinophil counts were normal, and this is in agreement with Gil FG et al as they reported cases without eosinophilia [55]. Stool examination had shown negative fluke eggs for all of the cases, as positions positive results need frequent sampling

[11], most of positive samples were from outside of Sulaimani and not easy to repeat the sampling for them. Liver function tests (AST, ALT, TSB, and ALP) done and nothing significant found, possibly due to the fact that the cases were early cases of Fascioliasis and also due to the lack of significant hepatobilliary obstruction. Histopathology results of the gallbladder of each patient have only shown features of chronic inflammation without cellular dysplasia or metaplasia.

5. CONCLUSIONS AND RECOMMENDATIONS

It is appearing that Fascioliasis is a zoonotic problem in our region, so it must be put in differential diagnosis in hepatobiliary problems especially gall stone diseases. The clinicians must aware about it, because any delay in diagnosis will lead to more complications. Fascioliasis is more common in Sharazur and spread to other areas through contaminated vegetables and drinking water.

It is a public health issue, so people working there must give awareness to society about this disease which is more easily preventable than to diagnose and treating it. In addition there are WHO recommendations for prevention of transmission of the disease and better to follow it.

In the view of what has been mentioned so far in this study, it would be a medical bonus to send bile samples from any suspicious patients undergo any hepatobiliary surgery, as this will improve the chance for detecting any Fasciola eggs and thus early meticulous diagnostic tool.

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