Anatomical patterns of intrahepatic cystic echinococcosis in reference to serological and clinical findings

Original Article

Mona S El-Sherbini[1], Ahmed Badawi Yousif[2], Sohier A Ismail[3], Amr M Abdelraouf[4], Iman R Abdel-Shafi[1]

Departments of Medical Parasitology[1,2], Hepatology and Gastroenterology[3], and HBP Surgery[4], Faculty of Medicine[1,2], Cairo[1] and Fayoum[2] Universities, and National Hepatology and Tropical Medicine Research Institute[3,4], Egypt

ABSTRACT

Background: Cystic echinococcosis (CE) is a zoonotic infection that occurs worldwide, particularly in endemic areas in the Middle East, including Egypt. *Echinococcus granulosus* (*E. granulosus*) eggs can be accidentally ingested by human in contaminated food or drinks and reach primarily the liver forming hydatid cysts with reported predilection for the right lobe. However, the segmental orientation of hepatic echinococcosis in the light of the clinical presentation and serological findings needs further investigation as such correlation may carry additional clues to guide the therapeutic management and prognostic outcomes.

Objectives: The present study was designed to determine the relation of anatomical location of intrahepatic hydatid cyst to its development, activity, and host immune response; as well as its potential extrahepatic spread.

Patients and Methods: A total of 46 patients having liver hydatid cysts were evaluated. Intrahepatic cysts were categorized according to Couinaud's segments by ultrasound examination; and analysis was done regarding cyst size, cyst staging and activity, and extrahepatic spread, in addition to clinical features and patterns of IgG level using two serodiagnostic tests IHA and ELISA.

Results: Hydatid cysts were found in all segments with the exception of segment I. Active cysts were mostly found in segment VII (no. = 11; 47.8%), recording a large diameter size with a mean of 6.55 cm. Inactive cysts recorded statistically significant smaller diameters with a mean of 5.55 cm denoting cyst evolution and degeneration. Serum antibody level correlated significantly with radiological profile of cysts activity in the studied population. Finally, extrahepatic spread was observed in hydatid cysts involving all liver segments with the exception of segment V.

Conclusion: Hydatid cysts were found in liver segments II through to VIII. While cysts in segment VII demonstrated large sized active cysts with extrahepatic spread, cysts confined to segment V pose minimal risk for extrahepatic spread.

Keywords: Couinaud's segments; cyst activity; cystic echinococcosis; immunodiagnosis; intrahepatic cysts.

Received: 9 May, 2020, **Accepted:** 10 June, 2020.

Corresponding Author: Iman R. Abdel-Shafi,, **Tel.:** +20 1223331861, **E-mail:** irriad@kasralainy.edu.eg

Print ISSN: 1687-7942, **Online ISSN:** 2090-2646, **Vol. 13, No. 2, August, 2020.**

INTRODUCTION

Hydatid disease or cystic echinococcosis (CE) is a zoonotic infection caused by the larval stage of *E. granulosus*. Global distribution poses a serious health problem in endemic areas in the Middle East, including Egypt[1]. Millions of *E. granulosus* parasite eggs spread *via* the excreta of dogs which are the definitive hosts. Accidental ingestion of eggs in contaminated food or drinks carries the risk of human infection. Once ingested, hatched oncospheres travel through the mesenteric vein to the portal vein reaching the hepatic sinusoids; accounting for 70% of hydatid cysts parasite load in the liver. The right lobe is the most frequently involved part of the liver, usually the anterior-inferior surface; however, scanty reports address the specific anatomic predilection of such focal cystic lesions[2].

Single organ involvement with solitary cyst is a common finding in most CE patients[3]. Still, extrahepatic spread occurs when oncospheres pass through the liver to the systemic circulation. They form cysts primarily in the lung, and less commonly in the spleen, brain, or bones[4]. The multiplicity of cysts is thought to be linked to the infection dose and the number of oncospheres filtered through the liver and lungs[3].

Growth rate of hydatid cysts is slow reaching 1 cm during the first 6 months and up to 2-3 cm annually, with a remarkable life span that can survive for up to 53 years in humans depending on host tissue resistance[5]. They usually attain more than 5 cm in diameter when they cause symptoms. Hepatic CE may show various clinical manifestations ranging from cholangitis with

 DOI:10.21608/puj.2020.29300.1071

biliary ruptures, to accidentally discovered cysts with no prior symptoms[6]. Therefore, early diagnosis is crucial to prevent possible complications that may occur.

Radiological ultrasonographic (U/S) imaging guided by the WHO classification is considered the current gold standard for diagnosing human CE. This classification categorized hydatid cysts into 5 distinct groups from CE1 to CE5. The U/S appearance of hydatid cysts vary, from a simple aspect to a more complex one, relative to the stage of evolution and maturity[7]. But not all radiological categorizations are exclusive for CE as small cysts from CE1 or inactive hydatid cysts in CE4 - CE5 groups could be misdiagnosed as neoplastic or simple cysts. Whereas, large CE1 cysts with double line sign, multi-septated CE2 and CE3 with water lily sign or daughter cysts are considered as active cysts with exclusive radiological findings[8]. Validation of inconclusive U/S examination can be provided by computed tomography (CT) and magnetic resonance imaging (MRI) techniques[7].

Serology has an ancillary role in the diagnosis of CE. Commercially available serodiagnostic tests, including indirect haemagglutination assay (IHA) and enzyme-linked immunosorbent assay (ELISA) lack reliability as a single indicator of hydatidosis[9]. This can be attributed to the variability in sensitivity and specificity[9], absence of local strains for CE detection in specialized laboratories[10,11], and to cyst factors as location, activity, and intactness[12-15].

Hepatic CE retains its morbidity and mortality owing to silent and evolutionary cyst complications such as compression, rupture and leakage, extrahepatic spread, in addition to possible risks accompanying the surgical interventions[16]. Since the liver is divided into 8 functionally independent units, referred to as segments, each with its own vascular flow and biliary drainage[17], an understanding of the precise cyst localization may give clues for early prediction of prognosis and eventually proper management, thus possibly reducing CE morbidity and mortality.

The present study was designed to determine the impact of intrahepatic segmental localization of hydatid cyst on CE course of events; namely development, activity and host immune response; as well as extrahepatic spread.

PATIENTS AND METHODS

This observational study was conducted retrospectively on data records of hepatic CE patients referred to Surgery Department, National Hepatology and Tropical Medicine Research institute, Cairo, over a period of two years from February 2016 to February 2018.

Patients: Records of 46 patients showed that they were subjected to history taking, clinical evaluation, radiological, and laboratory investigations as part of their routine care. They were distributed as follows: 29 (63%) males and 17 (37%) females; their age ranged from 22 to 55 years with a mean of 37.61 years. Regarding patients' residence, 24 (52.2 %) were living in rural areas, while 22 (47.8%) in urban areas. Twelve (26.1 %) patients reported having contact with dogs, and 34 (73.9%) negated such contact.

Liver ultrasonography (U/S): Ultrasonographic examination was conducted for all patients having cystic hepatic lesions. The liver was examined in all various planes with screening of the whole abdomen.

Cyst localization and size: For intrahepatic localization of the cysts, Couinaud classification system was adopted. It divides the liver into eight independent functional units termed "segments". Couinaud's liver segments, I through to VIII, are numbered in a clockwise manner. The left lobe includes segments II to IV, the right lobe includes segments V to VIII, and the caudate lobe is segment I[17]. Various diameters were measured for each cyst.

Cyst classification and staging: Radiologically, hepatic CE was classified into five groups according to the WHO classification following that of Gharbi *et al*.[18]. CE1 consists of a pure fluid collection; an uncomplicated unilocular or mono-vesicular cyst. CE2 is a fluid collection with a split wall; floating detached endocyst membrane. CE3 is a cyst containing daughter cysts and septations with a predominant fluid component; honey-comb image. CE4 is a cyst with a predominant heterogeneous solid pattern with few daughter cysts. CE5 is a calcified non-viable degenerated cyst with reflecting thick walls. Cysts are divided into active, CE1 and CE2; transitional, CE3; inactive, CE4 and CE5[19,20]. In the present study, CE 1-3 were grouped as active and CE 4 and 5 as inactive cysts.

Laboratory investigations: For serodiagnosis of CE, IHA and ELISA serological techniques were performed for each patient. The IHA was performed according to the instructions of the manufacturer (Fumouze® ELITech Microbio, France)[21]. For test interpretation, IHA titre 1:80 was considered negative, 1:160: borderline and ≥1:320: positive. ELISA specific IgG was performed according to the instructions of the manufacturer (Euroimmun, Perkinelmer Company, Medizinishe Labordiagnostika AG, Germany)[22]. Regarding the test interpretation, a negative result was recorded with the optical density (O.D.) values <0.8, while a borderline result was considered with O.D. values ranging from 0.8 to 1.1, and a positive result with O.D. values >1.1.

Statistical analysis: The collected data were tabulated and statistically analysed. Descriptive statistics in the form of percentage (%), mean, mode and standard

deviation (SD) was performed, as well as analytical statistics including Student's T-test to study association between two quantitative variables. Statistical significance was set at *P* value <0.05.

Ethical consideration: The study was conducted according to the institutional and ethics committee guidelines. Data were collected from patients' records as described in the patient's section.

RESULTS

Localization, size, activity of cysts and extrahepatic spread: On the lobar level, the 46 cysts were distributed as follows: 25 (54.35 %) were in the right lobe, 18 (39.13 %) were in the left lobe and 3 (6.52%) were bilobar. On segment level, CE were distributed in all segments, with the exception of segment I, and were frequently found in segments IV and VII. Regarding the number of involved segments, 29 cysts (63%) were in a single segment, 16 (34.8%) were in 2 segments, and only one cyst (2.2%) occurred in 3 segments. Cyst size ranged from 4.1 to 10.7 cm; the mean diameter and SD in each segment are presented in table (1). The highest mean diameter 10 cm was observed in a cyst located in segment VII, as well as other segments. Regarding cyst stage and activity, 23 active and inactive cysts were respectively recorded. However, active cysts were mostly found in segment VII (no. =11; 47.8%), either alone or in combination with other segments. The size of the cyst in the active stage recorded a mean of 6.55±2.07 cm while cysts in inactive stages recorded significantly smaller diameter with a mean of 5.55±1.51cm (*P*-value <0.05), denoting cyst evolution and degeneration. Extrahepatic spread was observed in 14 cases (30.4%). In these patients, cysts were detected in all liver segments with the exception of segment V (Figure 1A).

Clinical manifestations of CE: There were variable clinical presentations among the patients. The most common presentation was abdominal pain in right upper quadrant in 28 patients, recorded in cysts involving segments II through VIII. Other reported symptoms included urticaria in 4 patients having cysts in segments II, IV, VII and VIII. Interestingly, jaundice was identified in 4 patients, all of them having cysts involving segment VII with either segment VIII or V-VI. Biliary colics was reported in 2 patients having cysts in segments II and VII-VIII. In addition, asymptomatic cases (n= 9; 19%) were incidentally detected by U/S in segments III through VII (Figure 1B).

Serodiagnosis and cyst activity: The net number of positive cases recovered by ELISA was 37 cases and 24 by IHA, with sensitivity of 80.4% and 52.2%, respectively. For the ELISA and IHA results, the means of the O.D. and the modes of the titres respectively, are presented in table (2). The IHA results were treated as ordinal data due to the crude quantitative nature of the test based on serial dilution. The inactive cyst stages (CE4, CE5) reported negative or low positive values in both IHA and ELISA tests. Statistical significance was found between the ELISA O.D. in the active and inactive cysts groups, denoting that serum antibody level correlates significantly with radiological profile of cysts regression of activity in the studied population.

Table 1. Correlation between CE cyst anatomical site (Couinaud's segments), cyst size and activity by U/S in the studied population.

	Lobe	Segment	No.	Size	U/S classification				Activity	
				M ± SD	CE1	CE2	CE3	CE4	CE5	Active	Inactive
Cyst location	Lt. (No.=18)	II	3	5.88 ± 2.46	-	1	-	2	-	1	2
		III	6	4.76 ± 1.13	1	2	1	2	-	4	2
		IV	7	4.85 ± 1.54	1	-	3	2	1	4	3
		II-III	1	5.25 ± 0.78	-	-	-	1	-	-	1
		III-IV	1	4.05 ± 0.49	-	-	-	1	-	-	1
	Rt. (No.=25)	V	4	4.44 ± 0.66	-	1	-	2	1	1	3
		VI	2	4.40 ± 0.60	-	-	-	1	1	-	2
		VII	5	6.24 ± 1.25	1	2	1	-	1	4	1
		VIII	2	4.38 ± 0.88	1	1	-	-	-	2	-
		VII-VIII	5	7.47 ± 1.53	1	-	1	3	-	2	3
		VI-VII	4	7.35 ± 1.44	1	1	2	-	-	4	-
		V-VI	1	5.95 ± 0.35	-	-	-	1	-	-	1
		V-VIII	1	7.00 ± 0.00	-	-	-	-	1	-	1
		V-VI-VII	1	10.0 ± 1.41	-	1	-	-	-	1	-
	Bilobar (No.=3)	IV-V	2	4.08 ± 1.72	-	-	-	1	1	-	2
		IV-VIII	1	3.60 ± 0.85	-	-	-	-	1	-	1
	Total		**46**	**5.61 ± 0.55**		**6**	**9**	**8**	**16**	**7**	**23**

M ± SD: Mean ± standard deviation

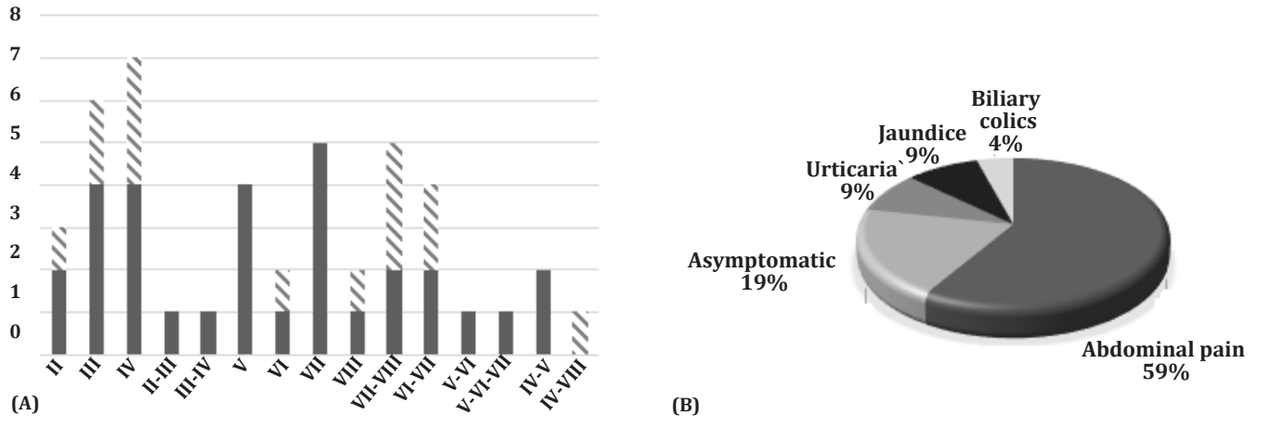

Figure 1. Extrahepatic spread and clinical manifestations of CE.
(A): The frequency of extrahepatic spread in cysts located in each liver segment.
(B): The percentage of various clinical presentations described in the studied population

Table 2. Relation between cyst activity and serological results of the studied population.

Activity	ELISA			Statistical analysis	IHA		
	Result	No.	OD (Mean±SD)		Result	No.	Titre
Active (No. =23)	Positive	22	3.1068±1.1095	*P* **value <0.05***	Positive	17	1:1280
	Borderline	1	0.9876		Borderline	5	1:160
	Negative	0	-		Negative	1	1:80
Inactive (No. =23)	Positive	15	2.3497±0.8334		Positive	7	1:320
	Borderline	6	0.9877±0.1062		Borderline	2	1:160
	Negative	2	0.6998±0.0304		Negative	14	1:80

ELISA: Enzyme-linked immunosorbent assay; **OD**: Optical Density; **M±SD:** Mean±Standard deviation; **IHA:** Indirect haemagglutination test; * Significant

DISCUSSION

Forty-six hepatic CE were described in the present study, with significantly high number of cysts found in the right lobe. Zhang *et al*.[23] succeeded to establish an animal model by intravenous injection of hydatid scolices in mice and reported the occurrence of the cystic lesions in all hepatic segments without significant lobe preference. However, the anatomy of human liver is different from that of mice as mentioned by the previous authors. Human liver is characterized by a bigger right lobe and a bigger wider right portal vein than the left side. In humans, the superior mesenteric vein joins splenic vein forming the portal vein which carries most of the infectious agents, including echinococcal eggs, to the right lobe of the human liver[15,24]; as in the case of the present results where the majority of the CE lesions were found in the right lobe. Consequently, due to anatomical structural and functional properties of the liver, our focus was to study CE not only at the liver lobes level but to go beyond that into the liver segments level. Excluding segment I, hydatid cysts were found in all liver segments with the highest number, mean diameter and activity of CE related to segment VII. In our study with the exclusion of segment I hydatid cystic lesions were found in all liver segments, with the highest number, mean diameter and activity related to segment VII. Basically, Couinaud's description of the liver segments is founded on the pattern of the hepatic vascular anatomy; where the right and left portal vein branches interdigitate with the right, middle and left efferent hepatic veins[25]. However, to date, and to the best of our knowledge, limited or no reports existed to explain the anatomical significance with the functional activity of hydatid cyst in specific hepatic segments.

Generally, extrahepatic spread results from exophytic growth and/or hematogenous dissemination. The former causes migration of cysts into the lungs, mediastinum, heart, and peritoneum, while the latter involves all other organs. Involvement of spleen was found to be the third most common location after liver and lungs in some case series. In these cases, it is commonly secondary to hematogenous dissemination as previously mentioned or intraperitoneal spread from ruptured liver hydatid cysts, while isolated primary splenic CE is rare[26,27]. Interestingly, in Polat and Atamanalp[28] study, the authors stated that trans diaphragmatic migration with lung invasion is most common in hydatid cysts located in segments VII and VIII due to their proximity to the diaphragm; while mediastinal and cardiac involvement is associated with liver segments II and IV. The absence of direct

or extrahepatic spread of hydatid cysts localized in segment V in our study is an observation that invites further investigations, to explain the micro-anatomical features of the liver in relation to hydatid disease.

The most common presentation in our cases was abdominal pain, while incidental diagnosis was reported in almost one fifth of the study population. Allergic manifestation was a minority in addition to some cases who suffered from complications as jaundice and biliary colic. This is in accordance with Rukmangadha *et al*.[29] and Salama *et al*.,[1] who reported that pain in the right upper quadrant was the most common symptom in hepatic CE. Nonetheless, Khader Faheem *et al*.[30] reported that the most of their patients were asymptomatic. Also, Akbulut[31] reported that only a smaller proportion of CE patients may become symptomatic and show complications such as cysto-biliary communication. Other clinical findings detected in our study included allergic manifestations, biliary colic and jaundice. Yet the latter complication was exclusively reported in CE lesions involving segment VII, that harboured the largest cyst with a mean size of 10 cm. Biliary complications are reported with large cysts close to the hepatic hilum, due to bile duct compression or rupture of hydatid cysts into biliary tree[32]. In fact, Atli and colleagues[33] stated that cyst diameters > 10 cm and > 14.5 cm are independent clinical predictors of frank and occult intra-biliary rupture, respectively.

Currently, serologic tests are used to support the radiological diagnosis and for follow-up of CE cases, yet several factors can affect these tests results. For instance, the immunological response to CE tends to vary from one individual to another. In addition to host reactivity, there is the larval cyst conditions as the cyst integrity: intact, leaking or ruptured; also the location, number, viability and finally the technique used and test format[34-36]. In our present study, ELISA showed a significant higher sensitivity in detecting hydatid cases than IHA (80.4% and 52.2% respectively). This was actually expected, as low sensitivity of IHA was previously reported to reach less than 50% even in hepatic CE[13]. Nonetheless, IHA test is the commonly used serological test in our laboratories, thus exposing the infected patients to false negative results and possibility of unpredicted complications. Therefore, standardized ELISA, preferably using local strain for hydatid antigen preparation should replace IHA in our health institutes. This finding was similar to that of studies done by Olut *et al*.[35] and El-Shazly *et al*.[38], who reported lower sensitivity of IHA test than ELISA in diagnosis of CE.

Concerning the relation of serology to cyst activity, in our study inactive CE4 and CE5 recorded either negative results or the lowest antibody levels, while active CE1, CE2 and CE3 reported positive serological results. These results may be related to possible leakage from active cystic lesions raising the level of immunogenicity hence giving positive serological results, as previously stated by Barnes *et al*.[39]. The negative sero-reactivity reported with inactive CE4 and CE5 stages may be explained by the study of Petrone *et al*.[40] who reported that a weak immune response occurs due to degeneration of the germinative membrane and calcification of cysts. Consequent to loss of fertility and degeneration antigens release is stopped resulting in low/absent immune stimulation. Several studies in turn reported that negative sero-reactivity is observed in up to one fifth of patients with CE, and its rate is relatively higher in patients with inactive CE4, CE5 cyst types as well as CE1. Moreover, patients with CE2 and CE3, and those with multiple cysts especially with multiorgan involvement are usually seropositive and may continue to remain so for more than 10 years despite treatment technique used and test format[35,36].

In general, hydatid infection is facing diagnostic difficulties by the existing antibody detection assays for the differentiation between past cured or calcified cysts from present active or progressive ones. In fact, implementation of serological tests may vary in different clinical stages of CE as reflected by the various radiological profiles of the WHO classification[34]. Therefore, the results of serological tests should be linked to other diagnostic tests as imaging methods, and negative serological tests do not exclude a hydatid cyst infection[13].

In conclusion, the present study was performed as a trial to determine some important CE parameters, namely the site, size, and activity of cysts in addition to antibody level in relation to specific hepatic location. Our results revealed three segments with distinct findings; segment I which was completely free of cysts; segment V in which no extrahepatic spread was recorded possibly posing minimal risk for such complication; and segment VII which demonstrated large active cysts with extrahepatic spread. Finally, this study reflects the importance of analysing all diagnostic and clinical finding in collaborative fashion; to conclude adequate predictive factors of CE prognosis which can affect management plan and spare the patients from possible complications.

Author contribution: El-Sherbini MS conceived the research idea, and shared in data interpretation, drafting and final editing. Ismail SA designed the study. Yousif AB and Abdelraouf AM collected the data. Abdel-Shafi IR analysed and interpreted the data and finalised the editing.

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