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Research Article

Fertility and Viability of the Hepatic Hydatic Cysts in Tunisian Patients

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Abstract

Cystic echinococcosis (CE) or hydatid cyst remains in Tunisia a major public health problem because of its high incidence and its considerable socio-economic repercussions. The diagnosis of hydatidosis, guided by the epidemiological context and the clinic, is based on imaging and serology. Our study proposed to investigate the relationship between the iconographic types of hydatid cysts and their fertility and viability. The study involved 80 patients operated for hydatid cysts of the liver for which we collected the operated cysts (82 cysts). Each sample of hydatid material was assessed for fertility (by protoscolex count) and viability (by microscopic examination and eosin staining). Our results showed that human hydatid cysts were very frequently fertile (73/82 hydatid cyst) with a high viability rate. Active cysts corresponding to type I and III cysts were generally more fertile than inactive cysts (type IV and V) and their scolex more viable.

Keywords: Cystic Echinococcosis; Fertility; Viability; Tunisian patient; Surgery

Introduction

Cystic echinococcosis (CE) is a worldwide zoonotic infection caused by the larval stage of cestode, *Echinococcus* (E.) *granulosus*. It has a major impact on public health with serious socio-economic consequences [1,2].

In humans, CE presents usually symptoms associated with the presence of cysts in the liver (the most frequently affected organ), lungs or other viscera. Asymptomatic forms are not uncommon [3,4].

In Tunisia, CE is endemic and considered as a major public health problem, especially in rural areas [5,6].

According to hospital statistics, the annual surgical incidence is 15 per 100,000 inhabitants per year. According to epidemiological investigations, serological prevalence and/or ultrasonographic ranged from 0.36% to 4.3% according to region [2,7].

The diagnosis of CE is generally established by a combination of imaging and serology. Serology confirms, if positive, the hydatid nature of the images detected by imaging and revealed hydatid nature of a tumor when iconography is doubtful. It also has a considerable contribution in the post operative follow up as repeated serological testing can detect the possible persistence or recurrence of cysts [8,9].

The aim of our study was to assess the relationship between the fertility and viability of liver hydatid cysts collected from tunisian patient after surgery, with reference to radiological aspects of the cysts and the study of their viability and fertility after surgical removal.

Material and Methods

Material

Human hydatid cysts. Our study is a prospective one. It was carried out in the laboratory of Parasitology, Farhat Hached

teaching hospital, Sousse, Tunisia. The study material was composed of 82 hydatid cysts of liver collected from 80 patients divided into 55 women and 25 men and aged from 11 to 85 year and operated for hepatic hydatid cycts in the surgery service of Sahloul university hospital, Sousse, Tunisia (48 patients) and Farhat Hached university hospital (32 patients).

For each cyst, the localization and the fertility of the metacercaria as well as age, sex and origin of the patient are listed.

The 82 operated and recovered hydatid cysts were transported to the laboratory of Parasitology on the same day or the next day after surgery. In the latter case, they were conserved at + 4°C.

The recovered hydatid material consisted of excised and formed by the hydatid membranes (number = 76) and/or the hydatid fluid and/or the entire cyst (number = 5), and, depending on the ease of exeresis and a risk incurred. Hydatid fluid was punctured after injection of the scolicide solution and hydatid membranes were collected separately in a sterile material. Whole cysts were collected into vials with 0.9% sterile physiological serum.

Methods

Extraction of hydatic material: For cysts, Hydatic liquid was aspirated with a sterile syringe. Eventual protoscolex to settle in fons after a few minutes. Hydatid material is removed completely after the opening of the cyst and treated as follows:

- When the parasite material consists of all or fragments of hydatic material, the product is rubbed and washed several times in a sterile solution of sodium chloride at 0.9%. The liquid is filtered through sterile gauze to remove large debris. The filtrate obtained is decanted 5 min at room temperature in sterile conical tubes.
- Three washes with 0.9% sterile sodium chloride followed by sedimentation of 1 min at room temperature are then carried out in order to maintain the majority of the living protoscolex. The supernatant of the last wash is finally removed and the pellet divided into aliquots of 100 uL in cryotubes, which are stored at -20°C until use.

Fertility of hydatid material

50 µL of the fluid sedimentation pellet that was used for washing of hydatid material was deposited and examined directly under the microscope (Figure 1).

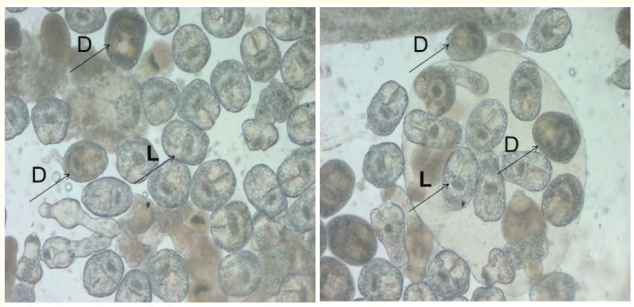

Figure 1: Direct examination of pellet of the washed hydatic membranes showing living protoscoleces (L) that appear to have a normal structure calcareous corpuscles and dead ones (D) that looks darker, with alteration of their structures (x100). Laboratory of Parasitology-Mycology, CHU Farhat Hached, Sousse, Tunisia.

Viability of protoscolex by staining

The viability of protoscolex was assessed by direct examination of scolices or a colorimetric test using the Eosin 1 ‰. After these 2 tests, the assessement of % of live protoscolex viability was assessed (Figure 2).

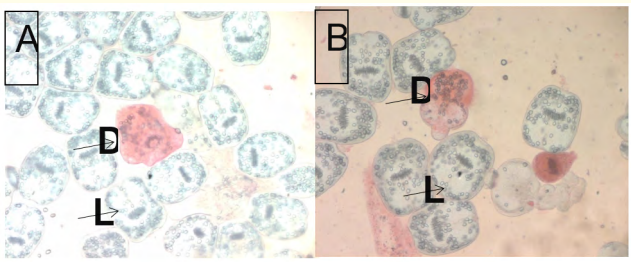

Figure 2 (A, B): Staining with 0.1% aqueous eosin solution showing dead protoscoleces (D) with a total penetration of the stain and living protoscoleces (L) with a preseved initial coloration, an ovoid form and intact calcareous corpuscles (x100). Laboratory of Parasitology-Mycology, CHU Farhat Hached, Sousse, Tunisia.

Study of fertility rate of hydatid cysts explored, percentage of viability protoscolex in relation with the aspect, activity, topography, size and number of HC, the existence of a cysto-biliary fistula, the age and sex of the patient.

The fertility of cysts and the viability of scolices was based on the following parameters:

- Type of the cyst according to the classification provided by surgeons.
- Active or inactive nature of the cyst according to the new WHO classification.
- Topography of cysts inside the liver.
- Size, number of hydatic cyst in the same patient.
- Presence or absence of kystobiliaire fistula.
- Age and sex of the patient.

Statistical analysis

The data were coded and entered into Epi InfoTM 6.04 software (Centers for Disease Control and Prevention, Atlanta, Georgia, USA). The statistical significance was accepted at the 5% level of significance and a 95% confidence level.

Results

The relationship between fertility and viability of cysts explored based on the parameters studied is summarized in the summary table I (Table I).

Relation Number		Fertility of explored cysts				Viability of explored cysts			
		Average of fertility (%)	Écart type	p	Number	Average of Viabiliy (%)	Écart type	p	
Surgeous classification	Type I	28	1773.5	1991.9		27	39.7	34.9	
	Type II	30	280.0	582.5		26	18.0	27.3	
	Type III	15	166.8	508.5	$< 10^{-4}$	8	18.6	32.0	0.03
WHO classification	Actif	64	956.5	1568.3		58	27.5	32.0	
	Inactif	15	166.8	508.5	$< 10^{-4}$	8	18.6	32.0	0.46
Topography cysts	Foie droit	42	930.5	1477.3		39	26.3	31.4	
	Foie gauche	22	788.2	1690.2		15	39.6	38.8	
	Segment I	2	30.0	21.2	0.70	2	0.0	0.0	0.20
Cyst size					0.16				0.45
Number of hydatid cysts in the same patient	1	52	910.7	1664.6		43	27.3	33.0	
	2	20	653.3	1067.4		17	21.6	29.7	
	3	4	192.5	228.6		3	13.5	11.6	
	4	2	412.0	229.1		2	15.8	4.8	
	5	1	235.0	//	0,25	1	0,0	//	0.80

Relation Number		Fertility of explored cysts				Viability of explored cycts			
		Average of Fertility (%)	Écart type	p	Number	Average of viability (%)	Écart type	p	
Presence of cysto-biliary fistula	Presence	23	453.2	822.2	0,18	18	12.9	21.3	
	Absence	58	924.0	1614.2		50	30.6	33.5	0.04
Age of the patient					0,76				0.19
Sex of the patient	Man	25	489.7	792.2		23	28.6	36.8	
	Women	55	941.2	1654.7	0,19	45	25.2	29.3	0.78

Table I: relationship between fertility and viability of cysts explored based on the parameters studied.

Discussion

In humans, the study of infectious protoscolex power remains essential to prevent the diffusion of scolex and secondary hydatid formation, major operative risk following the accidental spinning of protoscolex in the abdominal cavity [10].

Fertility of the cysts is a key factor in the transmission of parasite between the intermediate and the definitive hosts [10,11].

Studies on the fertility and viability of cysts are few and the results varies from one series to the other because of the non-standardization of methodologies applied to the study of these parameters [10-12].

In contrast to what is often admitted, our study showed that human hydatic cysts are met offer fertile as only 9 cysts (10.9%) out of the 82 examined were sterile, and the majority were also hyper fertile.

Indeed, in 2 studies of human cysts of Manterola., *et al*. and Garcia., *et al*, fertility were 57.1% and 46.2%, respectively [10,11]. Dueger., et al. found a percentage of 41.1% [12].

These differences may explain the process used in the counting of protoscolex. In our study, the ascertainment even a single scolex, whether viable or dead, to conclude in fertile nature of the cyst, while in other studies, the fertility level was estimated as less were considered fertile cysts or % of scolex living relative to the total of scolex was greater than or equal to 50% [13,14]. These discrepancies may also, at least in part be related to the conditions of sample conservation, deadlines of cysts exploration after surgical excision, the methodology.

Our study allows us to conclude that the larva of Taenia seems well adapted to humans although it is an accidental intermediate host and parasite is a impasse in the majority of cases.

Study of fertility according to the type of cyst

Our study revealed that hydatid cysts of type I were more fertile than hydatid cysts of type III and IV; This result is consistent with those of some authors where type I is often considered the highest risk of infection since it is most fertile [11]. However, for Martonela., *et al*, multivesicular cysts (type III) were more fertile than the cyst types I and IV, this is probably explained by a methodological differences protocols [10,14].

Study of the fertility according to the active or inactive state of the cysts

Based on the WHO classification, we found that the active cysts were more fertile than the inactive cyst, which is in agreement with the definition of WHO.

Study of the fertility according to the topography of the cyst

In contrast to Manterola., *et al*. where the fertility of cysts was significantly higher for cysts of the liver left lobe (58.2% Vs 50.0%), the cysts fertility rate in our series was independent of their topography [10]. The average fertility ralias of the right liver and left lots hydatic cysts and hydatic cysts those of the left liver were very close. The absence of significant binding can be explained by the predominant localization of cysts explored right liver from the left liver.

Study of fertility and the existence of a cysto-biliary fistula

Manterola., *et al*. have noted a statistically significant relationship between the fertility of the cyst and the presence of biliary communication; bile appearing to have an inhibitory effect on the proliferation of scolex inside the cyst [10].

In our series, despite the absence of statistically significant relationship between these two parameters, the average fertility of cysts with biliary fistula was half as compared to cysts without biliary communication.

The absence of significant correlation may be explained by the presence within the fistula diagnosed in intraoperatively, of small fistulas that quickly clogged by folds of hydatid membranes, leaving not spend bile, therefore no effect on the KH content and fertility.

Study of Fertility cysts according to the size, number of cysts, age and sex of the patients

In our study, no significant differences was found between fertility and these parameters. Indeed, the larger cysts were not necessarily more fertile; than the smaller of protoscolex were obtained from the cysts of small diameter, while cysts of large diameter were infertile. The most fertile cyst (7700 scolex) was 7 cm long axis. These findings are similar to those reported in the literature [15-17].

In our study, no statistical difference was observed between fertility and the number of cysts carried by the same patient; this result is similar to that found in other studies [10].

In addition, the same patient can harbour both fertile cysts and other low or non-fertile cysts in the same time. Furthermore, age and sex of the patient did not appear to influence the fertility of cysts; we observed highly fertile cysts in adolescents while some cysts were completely infertile some older subjects (> 50 years). Furthermore, the female predominance of hepatic hydatid disease seems to have translation in fertility of hydatic cyst. The results of these two last parameters are consistent with the literature data [10,16]. It therefore seems that the mechanisms regulating fertility are more complex than simple considerations of size and number of cysts, patient age or gender and are probably related to other factors related to the host or parasite.

Study of the variability of protoscolex

In our study, the percentage of viability of protoscolex from fertile cysts ranged from 0 to 99%.

In 17 cases, the percentage was null, all scolex being dead. These 17 cysts, only 3 had a rock water content; the content of the 14 remaining hydatic cysts was either infected or infected bile and is gelatinous and bilious. These conditions are considered a hostile environment for the protoscolex followed.

Study of the viability according to the type of cyst

According to our results, protoscolex were more viable in Type I cysts than in type III. This difference in viability was not observed between Type IV cysts or cysts between Type III and IV, although the type III cysts appear more viable than Type IV cysts. It has been shown that Type I cysts protoscolex bathed in hydatid liquid could remain alive for long periods [10,18].

The low viability of hydatid cysts of type IV has also been demonstrated by other authors.

In the study of Manterola., *et al*, hydatic cysts type III were more viable than type I cysts, but this finding needs to be verified because of the reduced number of the sample examined [14]. The higher viability of hydatic cysts type I is most likely related to the young age of the cysts. All of these results needs to be supported by additional tests such as culture of scolex, the electron microscopic examination and injection into mice.

Study of viability of protoscolex according to the presence or absence of a cysto-biliary fistula

As for fertility, we observed the same inhibitory effect of cysto-biliary fistula on the viability of protoscolex. This result is similar to that Thompson., *et al*. who demonstrated that the bile was harmful to the maintenance of scolex in culture whereas previously bile was used as a pretreatment to the evagination protoscolices in culture [19].

Study of the viability according on the activity, size, topography and the number of cysts on the one hand and the age and sex of patients the other hand

There were no statistically significant between the viability of protoscolex and these parameters. These finding needs to be considered with caution since the use of a scolicide intra operatively and the conservation of hydatid membranes in saline-sometimes overnight at 4°C can interfere with the percentage of viability of protoscolex.

Conclusion

In conclusion, human hydatid cysts are very frequently fertile. We have shown that the percentage of viability of scolex can be high, despite the use of hypertonic saline serum as scolicide. Overall active cysts are more fertile than inactive cysts and their protoscolex more viable.

Conflict of Interest

The authors have declared that no conflict of interest exists.

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None

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