Effect of some species of bacteria on viability of human hydatid cysts

Ismail Abdel –Wahab Al –Hadithi* ***Maisam Balasm Al-Khamesi*****

Mushtak TalibAl-Ouqaili**

Received 1, December, 2008
Acceptance 1, July, 2009

Abstract:

A total of 50 fertile human hydatid cases {33(66%) females and (34%) males}, obtained from Al-Ramadi public Hospital during the period from December 2003 to July 2004 were examined to study any bacterial infections. The specimens were obtained from hydatid fluid and then cultured on appropriate culture media to distinguish some species of bacteria which resulted in obtaining: *Staphylococcus aureus* (18%), *Pseudomonas aeruginosa*(12%), *Escherichia coli*(6%) and *Streptococcus pneumonia* (4%).

These bacteria were confirmed by isolation from interacyst fluid and blood culture technique. The possible routs of infection may be through blood, biliary ducts and bronchioles .The selectivity permeable of the cyst wall may be absent and that may allow some species of bacteria to enter inside the cyst.

Further, the percent viability decreased among cyst which yielded *S. aureus , P. aeruginosa* and other bacteria isolated after culturing compared with those of negative culture .Besides, the two types of protoscoleces motilities (flame cell activity and constriction –relaxation movement)increased in cases of negative culture .This association holds true at three different temperature (25ºC,37ºC and 40ºC).

Key words: Viability, Bacteria, Temperature, Hydatid cyst.

Introduction:

Hydatid disease was recognized as one of the world major zoonosis having a wide geograghic distribution [1,2]. It is still represents an important medical and sanitary problem in any regions and a challenge in common surgical practice world – wide [3].

It is well known that, hydatid cysts enter the body through the portal venous radicles and this haematogenous route is the primary route of entry and dissemination of hydatid disease [4].

It is a parasitic infection caused by the larval stage of *Echinococcus granulosus*, which is the causative agent of cystic hydatid disease or cystic echinococcos that can involve various organs [5,6].

It is well accepted that bacterial infection of hydatid cysts could be considered as another hazardous riskey factors , in addition to other complications usually associated with this serious parasitic infection in man [7,8].

[9] stated that bacterial infection of a cyst may occur, which can lead to a rapid increase in size, or to destruction of endocyst making it difficult to peal the cyst intact from its adventitia, perhaps causing a rupture of the hydatid cyst during the surgical intervention and dissemination of the

*Department of Applied Science, Biochemical Technology, University of technology, Baghdad, Iraq.
**College of science for women, Baghdad, Iraq.
***College of Medicine, Al-Anbar University, Al-Anbar, Iraq.

parasite elements, thus may lead to secondary echinococcosis. In addition to that, [10], mentioned that hydatid cysts may become sterile due to action of secondary bacterial infection, or they may die and become calcified.

The aims of the study to investigate some of the microorganisms that may be present in the hydatid fluid and their relationship to the cyst viability .

Materials and methods :

A total of 50 fertile hydatid cysts were obtained from 33 females and 17 males from Ramdi general hospital during the period from December 2003 to July 2004. The specimens were taken from different ages and site of infection 40 liver, 5 lung, 3 kidney and 2 urinary bladder.

Hydatid materials which includes fluid and cysts whether intact or ruptured were transported as soon as possible to the microbiological laboratory inside closed sterile containers . On the other hand the age , sex and site of cyst for all patients were recorded .

Each specimen of hydatid materials was cultivated on nutrient agar, blood agar , Chocolate agar and MacConkey agar after sterilizing the area of intact cyst with 70% alcohol and aspiration by sterile disposable syringe . After 48 hrs of incubation positive cultured specimens were examined while negative cultured were furtherly incubated for other 24 hrs .

Gram stain was used according to [11,12] coagulase test affer [11], oxidase test affer and IMViC test [13] were used as confirmatory test. which include Indole test , Methyl red test, Vogas proskaeur test and Citrate utilization tests, in addition to aspiration the blood from patients for blood culture technique.

Statistical analyses were compute assisted using SPSS (Statistical Package for Social Sciences), while in protoscoleces motilities the median used instead of mean to present the central tendancy of data & non-parametric tests will be used to assess the statistical significance.

Results and discussion:

Table 1 indicates that 60% of the samples showed negative results in culture .The positive results obtained in remaining 40% of cases and they were as follows ;

Staphylococcus aureus (18%) ,
Pseudomonas aeruginosa (12%),
Escherichia coli (6%) and *Streptococcus pneumoniae* (4%).

Two kinds of motility were seen, flame cell activity and constriction – relaxation activity (invagination-evagination movement) .One way of describing motility is noting the total absence of both types of movement in all examined protoscoleces.

The rate of cases with immotile protoscoleces was obviously higher (100%) among cases with clinical evidence of infection compared with those with no infection (68.1%). The evidence however failed to reach the level of statistical significance (Table 2).

The rate of cases with immotile protoscolesces was also higher (100%) among cases which receive medical treatment prior to surgery compared with those with no pretreatment (67.4%)

The isolated of *S. aureus* and *P. aeruginosa* increased the risk of having totally immotile protoscoleces by 6.1 and 3.8 times respectively compared to those with negative culture which failed also to reach the level of statistical significant . In these cases the presence of some bacterial species inside the hydatid cyst may indicate the absence of selectivity permeable of the cyst wall, this result was similar to [14].

Evidence of the absence of any sign of viability of the protoscolesces after staining was documented to clear a dead cyst .The rate of cases with dead cyst was significantly higher (100%) among cases with clinical evidence compared with those with no infection (23.4%)

The rate of cases with dead cyst was also higher (75%) among cases which received medical treatment prior to surgery compared to those with no pretreatment (23.9%) (Table 3) .The median percent of viability decreased from as high 46% among negative culture to 28% ,25% and 0% among cyst which yield *S. aureus* ,*P. aeruginosa*. and other bacteria respectively after culturing .This negative associated between the presence of bacteria ands viability was statistically significant (Table 4).

The median percentage of protoscoleces with flame activity or constriction-relaxation movement was significantly higher in the group with negative culture compared to those with *S. aureus* , *P. aeruginosa* and other bacteria isolated from culture media .This association holds true at 3 different temperatures (25ºC,37ºC,and 40ºC). It is worth noting that the median percentage of protoscoleces with flame activity or constriction – relaxation movement increased steadily with rising temperature among the group with negative culture, while this positive trend was not observed among cysts in which bacteria were isolated from culture (Table 5).

In this study, the viability of human hydatid cyst showed high rate in high numbers of cases ,except many cases such as , infected hydatid cyst which contained small numbers of dead and shrinkage protoscoleces in addition to degeneration materials . These cases showed complete absence of any sign of viability (100%) due to presence of certain species of bacteria such as *S. aureus* , *P.aeruginosa* & other species which were isolated.Therefore, these cases also showed absence of any sign of viability in (75%) due to the effect of treatment on hydatid materials with Tinidazole, compared to those with intact cyst .

Statistical analysis showed no significant difference in pretreatment cases with viability of hydatid cyst, probably because of small sample size.

Secondary bacterial infection of the cysts can result in liver abscesses, the organisms recovered from liver abscesses are varied and often reflect the origin of the infectious process. Mixed facultative and anaerobic species are isolates most frequently [15,16]. When the biliary tree is the source of infection, enteric gram negative aerobic bacilli and enterococci are common isolates. Anaerobic are not generally prominent in liver abscesses arising from biliary infections since anaerobes are not common constituents of the gall bladder unless previous surgery or stenting of the biliary tree has been performed. While with hematogenous spread of infection, a single organism is most commonly isolated including *S. aureus* or a streptococcal species [17,18].

Our results revealed, presence and isolates of some species of microorganisms from the cyst wall and fluid like ;*S. aureus* ,*P. aeruginosa* ,*E. coli* and *S. pneumoniae* as confirmed isolation of bacteria from intracyst fluid and blood culture technique .This presence may be due to the absence of selective permeability of the cyst wall .The source of infection may be through blood or biliary ducts and bronchioles [1].

This study was supported by aspiration of blood from patients with hydatid disease that were suffering from bacteremia , and then cultured under sterile conditions .So, the large

numbers of cases which yielded a positive culture in appropriate culture media in vitro gave the same result after aspiration of the blood from patients with hydatid disease after surgical removal for blood culture .our result was similar to those obtained by [10].

In this study ,the presence and isolate of certain species of bacteria showed clearly effect of the percent of viability due to highly visible decrease of this percent .This negative association between the presence of bacteria and viability was statistically significant .In addition to the two types of motility like flame cell activity and constriction –relaxation it also showed more decrease in motility when compared to those with negative culture. This association holds true at 3 different temperatures (25ºC, 37ºC and 40ºC).

Table 1: Frequency distribution of the study samples by results of culture.

Results of culture	N	%
Negative	**30**	**60**
Staphylococcus aureus	**9**	**18**
Pseudomonas aeruginosa	**6**	**12**
Escherichia coli	**3**	**6**
Streptococcus pneumoniae	**2**	**4**
Total	**50**	**100**

N= number of cases

Table 2: The risk of absence of any kind of motility of protoscoleces by selected explanatory variables.

	Total	Absence of any kind of motility		
	N*	N	%	P
Clinical evidence of infected cyst				**0.54[INS]****
Negative	**47**	**32**	**68.1**	
Positive	**3**	**3**	**100**	
Pretreated cases				**0.3[INS]**
Negative	**46**	**31**	**67.4**	
Positive	**4**	**4**	**100**	
Results of culture				
Negative	**30**	**17**	**56.7**	
Staphylococcus aureus	**9**	**8**	**88.9**	**0.08[INS]**
Pseudomonas aeruginosa	**6**	**5**	**83.3**	**0.22[INS]**
Others	**5**	**5**	**100.0**	**0.08[INS]**
Isolation of bacteria in culture				**0.01**
Negative	**30**	**17**	**56.7**	
Positive	**20**	**18**	**90.0**	

*** Number of cases.**
**** Non Significant.**

Table 3: The risk of complete absence of any sign of viability of protoscoleces by selected explanatory variables.

	Total	Complete absence of any sign of viability		
	N	N	%	P
Clinical evidence of infected cyst				**0.02**
Negative	**47**	**11**	**23.4**	
Positive	**3**	**3**	**100.0**	
Pretreated case				**0.06[INS]**
Negative	**46**	**11**	**23.9**	
Positive	**4**	**3**	**75.0**	
Results of culture				
Negative	**30**	**3**	**10.0**	
Staphylococcus aureus	**9**	**3**	**33.3**	**0.12[INS]**
Pseudomonas aeruginosa	**6**	**3**	**50.0**	**0.04**
Others	**5**	**5**	**100**	**<0.001**
Isolation of bacteria in culture				**<0.001**
Negative	**30**	**3**	**10.0**	
Positive	**20**	**11**	**55.0**	

Table 4: The difference in median viability (relative frequency of viable protoscoleces) by result of culture.

	Percent viability		
	Range	Median	N
Negative	**(0 - 72)**	**46**	**30**
Staphylococcus aureus	**(0 - 75)**	**28**	**9**
Pseudomonas aeruginosa	**(0 - 68)**	**25**	**6**
Others	**(0 - 0)**	**0**	**5**
P (Kruskall-Wallis) = 0.001			

Table 5: The difference in median of Percent protoscoleces with flame activity- and percent of those with constriction relaxation movement by result of culture and temperature.

	Percent protoscoleces with flame activity-			Percent protoscoleces with constriction-relaxation movement		
	25 °C	37 °C	40 °C	25 °C	37 °C	40 °C
Negative						
Range	**(0 - 9.8)**	**(0 - 49.2)**	**(0 - 88.2)**	**(0 - 5.6)**	**(0 - 58.7)**	**(0 - 100)**
Median	**1.8**	**31.8**	**64.9**	**0.5**	**44.5**	**100**
Number	**30**	**30**	**30**	**30**	**30**	**30**
Staphylococcus aureus						
Range	**(0 - 1.3)**	**(0 - 41.2)**	**(0 - 19.8)**	**(0 - 1.1)**	**(0 - 41.8)**	**(0 - 100)**
Median	**0**	**0**	**0**	**0**	**0**	**0**
Number	**9**	**9**	**9**	**9**	**9**	**9**
Pseudomonas aeruginosa						
Range	**(0 - 1.9)**	**(0 - 42.3)**	**(0 - 18.2)**	**(0 - 2.1)**	**(0 - 36.3)**	**(0 - 100)**
Median	**0**	**0**	**0**	**0**	**0**	**0**
Number	**6**	**6**	**6**	**6**	**6**	**6**
Others						
Range	**(0 - 0)**	**(0 - 0)**	**(0 - 0)**	**(0 - 0)**	**(0 - 0)**	**(0 - 0)**
Median	**0**	**0**	**0**	**0**	**0**	**0**
Number	**5**	**5**	**5**	**5**	**5**	**5**
P (Kruskall-Wallis)=	**0.003**	**0.006**	**<0.001**	**0.002**	**0.001**	**0.006**

References

1. Gottestein, B., Saucy, F., & Deplazes, P. 2001. High prevalence of *Echinococcus multilocularis* in wild & domestic animals associated with increased disease incidence in human. Emerg. Infect. Dis., 7: 408-412.
2. Mahmoud, S. S. and Al-Janabi, B. M. 1983. Hydatid disease in children and youths in Mosul, Iraq .Ann . Trop .Med .parasitol .,77:237-238.
3. Schants, P. M. 1999. Editorial response. Treatment of cystic echinococcosis. Clin. Infect. Dis., 2: 310-311.
4. Bouree, P. 2001. Hydatidosis: dynamics of transmission. World J. surg., 25: 4-9.
5. Galindo, M., Gonzalez, J. & Galanti, N. 2002. Echinococcus granulosus protoscolex formation in natural infection. In: Biological research. Faculity of Medicine, University of Chile, Santiago, Chile., 35: 365-371.
6. Gottestein , B. and Hemphill , A. 1997 .Immuonopathology of echinococcosis .Chem.Immunol.Basal.Karger.,66:177-178.
7. Brooks, G. F., Janet, S. B. & Morse, S. A. 2001. Medical Microbiology. 22nd ed. McGraw-Hill. Toronto.
8. Salinas, J. C. Torcal, J., Lozano, R., Sousa,R., Morandeira,A. and Cabezali ,R. 2000. Intracystic infection of liver hydatidosis hepatogastroenterology.,47:1052-1055.
9. Kilani, T., El-Hammami, S. & Horchani, H. 2001. Hydatid disease of the liver with thoracic involvement world J. surg., 25: 40-45.
10. Markell, E., Marietta, V. and David, J.1986. The Cestodes. In : Medical Parasitology. 6th ed. WB. Saunders CO.
11. Sood, R.1995. A color atlas of practical pathology and microbiology 2nd ed. Jayapee brothers medical publishers.
12. Alexander, S. K. and Strete , D. 2000. Microbiology atlas for the laboratory .Benjamin Cummings. Canada.
13. Baron, E. J. Peterson , L. R. and Finegold , S. M. 1994. Bailey

&Scotts diagnostic microbiology .9th ed ..Mosby Company.Toronto.

14. Al-Zubaidi, B. A. 1989. The chemical structure of hydatid fluid and laminated layer for *Echinococcus granulosus* from human and some intermediate hosts in Iraq. M.Sc. Thesis. College of Science, University of Al-Mosul.

15. Levinson, W. & Jawetz, E. 2000. Medical Microbiology & Immunology Examination & Board Review. 6th ed. McGraw-Hill. Toronto.

16. Fiorillo, L., Zucker, M. & Sawyer, D. 2001. The Pseudomonas hot-foot syndrome. J. Med., 345: 335-338.

17. Pollck, M.2000. Pseudomonas aeruginosa. In: Principles & practice of infectious disease. (eds. Mandell, G. L., Bennett, J. E., & Dolin, R.). 5thed.Churchill Livingstone. New York.

18. Ibrahim, E. H., Ward, S. & Sherman, G. 2000. A comparative analysis of patient's with early – onest VS late – onest nosocomial Pneumoniae in the LCV setting. Chest., 5: 1434-1442.

تأثير بعض الأنواع البكتيرية في حيوية الأكياس العدرية في الإنسان

اسماعيل عبدالوهاب الحديثي* ***ميسم بلاسم نعيم الخميسي*****

مشتاق طالب العكيلي**

* قسم العلوم التطبيقية /فرع التقانات الكيميائية الأحيائية /الجامعة التكنولوجية ، بغداد/العراق

** كلية العلوم للبنات /جامعة بغداد ، بغداد /العراق

*** كلية الطب /جامعة الانبار ، الانبار /العراق

الخلاصة:

فحصت عينات الأكياس العدرية وضمت 50 كيسا مائيا بشريا خصبا 33 (66%) من الأناث و 17 (34%) من الذكور، من مستشفى الرمادي العام للمدة الممتدة من كانون الأول 2003 الى تموز 2004، بهدف دراسة الأصابات البكتيرية ، وأخذت هذه العينات من السائل العدري ثم زرعت في وسط زرعي ملائم للكشف عن بعض الأجناس البكتيرية اذ تم الحصول على:

Staphylococcus aureus (18%) , *Escherichia coli* (6%) , *Pseudomonas aeruginosa* (12%) and *Streptococcus pneumoniae* (4%)

التي عزلت من داخل الأكياس فضلا عن تقنية زرع الدم . قد تكون هذه الأصابات دخلت عن طريق الدم ، القنوات الصفراوية والقصيبات ، او قد تكون النفاذية الأختيارية للكيس العدري مفقودة مما يسمح بدخول بعض الأجناس البكتيرية لداخل الكيس . قلت نسبة حيوية الرؤيسات الأولية في الأكياس التي كانت تحتوي على *S. aureus* , *P. aeruginosa*

واجناس اخرى عزلت ، مقارنة بنتائج الزرع السلبية . الى جانب ذلك فأن حركتي الرؤيسات الأولية (الحركة اللهبية و حركة التقلص - الأنبساط) ازدادت مع نتائج الزرع السلبية ، تزامنت هذه النتيجة مع درجات الحرارة الثلاث (25°م ، 37°م ، 40°م).