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Molecular Characterization of Bilateral Pulmonary Hydatid Cysts in a Child

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Abstract: A 14 year old female was admitted to Elshab Medical Teaching Hospital, Khartoum, Sudan. As informed by the father, there was a history of cough for 2 months, chest pain for 2 months and breathlessness for 3 weeks. The X-ray and MRI revealed the presence of bilateral pulmonary hydatid cyst. The patient was referred to thoracic surgery unit for thoracotomy of the left side of the chest. Because of the deteriorating condition of the patient, thoracotomy was initially performed in the right side of the chest to remove the giant hydatid cyst. A week later, thoracotomy was also performed to remove a ruptured pulmonary cyst from the upper lobe of the left lung, which was found infected with secondary bacterial organism. Molecular characterization rebelled that the cyst is of genotype 6 (G6) strain as detected by polymerase chain reaction (PCR)-based assay.

Key words: Molecular, pulmonary, hydatid cyst, bilateral

INTRODUCTION

Hydatid disease, caused by *Echinococcus granulosus*, affects humans and animals and hence the disease is of public health importance[1]. Cystic pulmonary hydatidosis in children or adolescence is very rare although it is commonly observed in adults. The mature worms of this cestode parasite are maintained in a carnivore definitive host whereas the larval stages are harboured by a herbivore intermediate host. The disease is prevalent in Africa including the Sudan, especially in rural areas where animal slaughtering is practiced on farms. In addition, improper disposal of offal from slaughterhouses and the presence of large populations of stray dogs could also contribute towards the endemicity of the disease[1,2]. The parasite causes serious public health problems in certain parts of the Sudan[1,3]. Humans can accidentally become infected by ingesting eggs of adult worms and hence, cystic hyadtidosis, caused by *Echinococcus granulossus,* is a disease of public health importance. Ten genotypes (strains) of *E. granulossus* have been identified worldwide, designated (G1 to G10). In a previous study, the camel genotype (G6) was believed to be the most prevalent strains of the parasite in the Sudan[4]. Despite the endemicity of cystic hydatidosis in African and Mediterranean countries, very little information is known in regard to molecular characterization of the genotype of this cestode parasite. In this study, we present an unusual case of bilateral pulmonary hyadtidosis with a ruptured cyst in the right lung and an infected cyst in the left lung. The pulmonary cysts were surgically removed and submitted to the diagnostic laboratory for further parasitological examinations and for identification of the genotype (strain) of the parasite using polymerase chain reaction (PCR)-based detection assay.

MATERIALS AND METHODS

Parasite: Hydatid cyst was obtained from a patient following surgical operation. Hydatid fluid was aspirated aseptically and hydatid cyst was surgically excised from the lung tissues.

Parasitological examination: Examination of the cyst content revealed the presence of Free living prtoscolices. The protoscolices were stained with eosin and viability was demonstrated under the microscope. Thus, the cyst was identified as hydatid cyst of the cestode parasite *Echinococcus granulosus*.

Extraction of nucleic acid from the hydatid cyst: The surgically removed pulmonary hydatid cysts were preserved in 70% alkahol and submitted to the molecular

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diagnostic laboratory for molecular characterization. The cysts were washed throughrouly with nucleic acid free water by centrifugation to remove the 70% alcohol. DNA was then extracted from protoscolices using QIAamp extraction kit. Briefley, the protoscolices were homogonized and the homogenate was lysed using lysing buffer (500 µL digestion buffer, 60 µL proteinase K, 10 µL DTT). The DNA was extracted with using QIAmp Briefly, (200 µL of the homogenate supernatant, 30 µL of proteinase K stock solution and 210 µL of lysing buffer were pipetted into 1.5 mL eppendorf tube and the mixture was incubated at 37°C for one hour and then at 70° C for 30 mins. 210 µL of absolute ethanol was then added to the sample and mixed by vortexing. The mixture was then transferred to the QIAamp spin column and placed in a clean 2 mL-collection tube and centrifuged at 8000 RPM for 1 min at room temperature. The QIAspin column was washed twice using 500 µL of washing buffer and spinning for 1 min. The QIAamp spin column was placed in a clean 1.5 mL epenndorf tube and the DNA was eluted with 200 µL of double distilled water preheated at 70°C at room temperature. Maximum DNA yields was obtained by spinning at 12,000 g for 1 min at room temperature. The DNA concentration was determined by spectrophotometer at 260 nm wave length. Five µL of the suspended nucleic acid was used in the PCR amplification.

Polymerase chain reaction (PCR): The eluted DNA was stored at -20°C untill used for PCR amplification. The polymerase chain recation (PCR) was employed to determine the starins of *E. granulosus* as described by[4]. Primers p1and p2 were used to amplify the 254 bp first PCR product from pig, cattle and camel strains (G 5, G 6 and G 7) respectively. Primers P1 and p3 were used to amplify the internal (semi-nested) 171 bp PCR product, which is specific for camel strain (G 6). PCR products were loaded onto 1.5% agarose gel and electrophoresed. The gels were then stained with ethidium bromide and the specific bands were vissualized under UV light.

RESULTS AND DISCUSSION

Parasitological examination of the right pulmonary cyst revealed the presence of hydatid cyst. Confirmation of hydatid cyst was made possible by demonstration of viable protoscolices under the microscope. The cyst was viable and fertile as free living protoscolices were observed under the microscope. Molecular characterization of hydatid cyst-derived protoscolices was made possible using G6-specific PCR-based detection assay. Using a pair of outer primers, the PCR assay produced a 254 bp specific PCR products from DNA samples extracted from hydatid cyst of the infected patient (Fig. 1). Using a pair of semi-nested primers, the PCR assay produced a 171-bp nested PCR product from the primary PCR product (Fig. 2). The primary and the nested PCR products were visualized onto ethidium

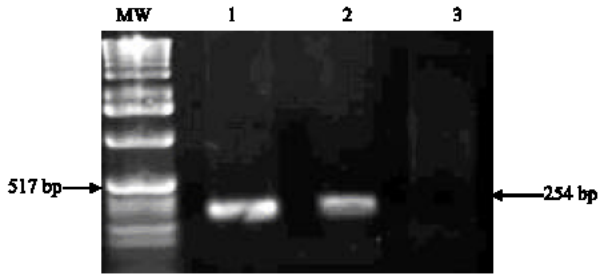

Fig. 1: Visualization of a 256 bp primary PCR product from DNA extracted from the patient hydatid cyst G1-specific outer primers

Lane MW: molecular weight marker (1Kb ladder); Lane 2: DNA from *E. granulosus* G1 (positive control); Lane 2: DNA extracted from protoscolices associated with patient's hydatid cyst. Lane 3: Negative control.

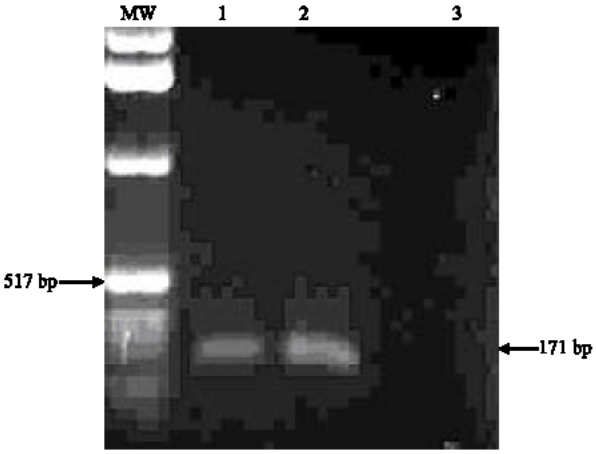

Fig. 2: Visualization of a 171 bp seminested PCR product from DNA of protoscolices associated with patient's hydatid using G1-specific seminested primers

Lane MW: molecular weight marker (1Kb ladder); Lane 2: DNA from *E. granulosus* G1 (positive control); Lane 2: DNA extracted from protoscolices associated with patient's hydatid cyst. Lane 3: Negative control.

bromide-stained agarose gels. Thus, the recovered hydatid cysts were identified as camel strain (G 6) of *E. granulosus*.

Hydatid lung disease caused by *Echinococcus granulosus* is often asymptomatic and usually benign. Surgical interference is not generally recommended unless the cyst is interacting with the normal function of the affected organ. Hydatid lung disease of adolescence in the African continent is usually caused by *E. granulossus*. Slowly enlarging *Echinococcus* cysts usually remain asymptomatic until their expanding size or their space occupying effect elicits symptoms. The cysts may be discovered as an incidental finding on a routine X-ray chest. Pulmonary hydatid cyst may rupture into pleural cavity, pericardium or the bronchial tree leading to cough, chest pain and haemoptysis. In the present case, the hydatid cyst, located at the lower left lobe of the lung, had ruptured and was invaded by secondary bacterial infection leading to empyema, which mandates surgery to remove the cyst[5,6,7]. Following this surgical procedure, the patient returned rapidly to Good health.

Diagnosis becomes difficult once the pulmonary hydatid cyst ruptures spontaneously or following trauma and gets secondarily infected. A more common cause of empyema is the infection of pleural fluid as reported in our case. It is worth mentioning that the general practitioner, who handled the case at the time of admission, did not investigate for hydatid lung cyst before insertion of intercostals drainage tube. This is probably due to the fact that one would not think of the disease in such young age group. Nevertheless, he did not attempt to obtain some information about previous history of contact with dogs. It is well documented that younger patients (children and teenagers) are more prone to be symptomatic and to develop complications from ruptured hydatid cysts. This is because children develop very large cysts in relation to the size of the lung (6-12 cm), due to greater elasticity of the lung. The cyst could also lead to a large residual cavity that takes more time to resolve and is then more likely to develop infections. It is well documented that the bronchial tree in younger patient is smaller than in adults and hence expulsion of membranes and particles is compromised.

It is worth mentioning that very little information is available in regard to genotypes of *E. granulosus* strain in the Sudan. Previous studies showed that the camel strain (G6) is the most prevalent strain of the parasite in humans and animals. Two cases of *E. granulosus* (G 5) were reported sporadically in a survey of the disease in Khartoum State. The camel strain (G 6) has never been reported in the sudan. In this study we report on isolation of G6 strain of *E. granulosus* in a child in the Sudan. From an epidemiological point of view, the patient is originally from Malakal area of the upper Nile Province of the Southern Sudan. Thus, it is probably that this strain (G 6) of the parasite is circulating in southern Sudan. However, additional data are needed to confirm this assumption. In this study we would also like to point out the importance of a good history, especially of contact with dogs in the patients who develop respiratory complaints spontaneously or following mechanical trauma to chest. Such patients should be investigated on the lines of pulmonary hydatid cyst so as to facilitate rapid diagnosis and subsequent successful treatment.

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