Human Cystic Echinococcosis with Special Reference to India — An Overview

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Abstract

Cystic echinococcosis (CE) caused by the cestode parasite Echinococcus granulosus is a significant public health problem in India, evident from widely distributed case reports and hospital based studies. The epidemiological reports are limited and the current prevalence/incidence is difficult to interpret. The prevalent age group reported depends upon the time of acquisition of symptoms rather than the period of actual transmission of infection, which is difficult to determine. The clinical manifestations may appear year’s later following infection and thus the age group preponderance may not be truly interpreted. The gender prevalence varies across different regions, probably attributable to differences in socioeconomic and cultural factors in these regions. The clinical features are variable, depending mainly upon organ involved and mimic many other diseases. Thus, the diagnosis of CE is complex and necessitates the use of clinical, radiological and immunological techniques. The conventional serodiagnostic techniques using crude specific antigens yield low specificity. The identification of diagnostic antigenic fractions aid in more accurate diagnosis, however, variable specific immunoreactive fractions have been reported from different geographical areas worldwide, which may be due to variations in the strain and source of cyst used for antigen preparation. Surgery is the choice of treatment while for inoperable cases medical treatment is advised. Molecular epidemiology studies have revealed presence of G1, G2, G3, G5 and G6 E.granulosus genotypes in north Indian patients. Intersectoral collaboration of medical, veterinary and agricultural sciences, along with integration of CE control program with other control programs is desired. This may reduce costs, improve participation and facilitate implementation of the program. Research thrusts to develop effective molecular and immunological methods for early diagnosis, identification of newer drugs with higher efficacy and develop an efficient vaccine for dogs and sheep have been suggested to provide a solid platform for effective control programs.

ABBREVIATIONS

CE: Cystic Echinococcosis; AE: Alveolar Echinococcosis; DNA: Deoxyribosenucleic Acid; PCR: Polymerase Chain Reaction; ITS: Internal Transcribed Spacer; RFLP: Restriction Fragment Length Polymorphism; FNAC: Fine-Needle Aspiration Cytology; ELISA: Enzyme Linked Immunosorbent Assay; IFA: Indirect Fluorescent Antibody; IHA: Indirect Haemagglutination; LA: Latex Agglutination; CIEP: Counter Current Immunoelectrophoresis; WB: Western Blot; Th1: T Helper Cell-1; Th2: T Helper Cell-2; PAIR: Puncture, Aspiration, Injection and Reaspiration; USG: Ultrasonography

INTRODUCTION

Echinococcosis or hydatid disease is of significant importance causing economic implications globally. It is included in the list of neglected tropical diseases and is considered to be one of the six priority neglected zoonotic diseases [1]. The infection is caused by cestode parasites of the Genus Echinococcus, the adults of which are found in carnivore definitive hosts. The definitive hosts shed eggs which are ingested by the intermediate hosts (including rodents, domestic and wild ungulates). Further, it develops to the metacestode stage and establishes a cystic, alveolar or polycystic mass in the body organs. Humans are accidental intermediate hosts and can be infected by ingestion of parasite eggs by direct contact with definitive host or through contaminated water and food sources. Two predominant forms of human hydatidosis are cystic echinococcosis (CE) caused by E. granulosus and alveolar echinococcosis (AE) caused by E.multilocularis. CE is the most frequent and AE the most aggressive form. E.oligarthus in Colombia and Panama, and E.vogeli in central and South America.
have been rarely found to infect man. In addition, *Eshigicus* and *Efelidis* have been found in Tibetan fox and African lion, respectively, however, their zoonotic transmission potential to humans is presently unknown [2]. In India, there are only a few reports of *E.multilocularis* infection [3,4] while two earlier reports of *Eoligarthus* were later termed as erroneous on the basis of morphology of the cyst and absence of host [5,6]. It was concluded that these were due to *E.granulosus* [7].

**EPIDEMIOLOGY**

The human infection by *E. granulosus* is nearly cosmopolitan and is estimated to account for more than 95% of about 3 million cases around the globe. It is estimated that 1–3 million disability-adjusted life years are lost per annum because of CE. The annual cost of treatment of cases and economic loss to the livestock industry is estimated to amount to US$2 billion [8]. CE is endemic in more than 100 countries globally. The prevalence of infection differs widely and is reported most commonly in countries where sheep and cattle raising are important thriving industries. The highest prevalence is found in temperate zones (Mediterranean regions, parts of Russia, central Asia, China), Australia, parts of South America and Africa. The annual incidence in endemic areas vary from <1 to 220 per 100,000 persons, while the mortality rate ranges from 2 to 16 percent [9]. The annual surgical incidence in endemic areas worldwide varies from 0.87 to 162 and in western and central Asia from 0.87 to 6.6 per 100,000 inhabitants. Although, it is postulated that the worldwide prevalence and incidence of CE has fallen dramatically in certain areas over the past several decades; yet it is emerging and re-emerging in areas previously with low level of infection [10].

CE is endemic in many regions in India, as is evident by hospital based studies [11-19] (Table 1), case reports [20-39], and seropositivity to hydatid fluid antigens in asymptomatic subjects [40], however, detailed epidemiological studies from

<table>
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<th>Geographical area(s)</th>
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<td>Hydatid cases-Age range 17-50 years; gender not significantly different</td>
<td>Fine needle aspiration cytology (FNAC) reports of 125 samples during a period of 3 years (2011-2013) from superficial and deep lesions revealed that 8 (6.4%) were hydatid cysts.</td>
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<td>Central and southern Andhra Pradesh, South India 2. Khader et al. [12]</td>
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<td>West Bengal, East India 3. Ghoshal et al. [13]</td>
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<td>Median age of presentation was 33 years; female preponderance</td>
<td>Analysis of 5 years data (2005-2009) revealed that 106 patients were diagnosed of pulmonary CE, based on clinical, radiological features, ELISA, surgery and outcome of treatment.</td>
<td>The surgical treatment is effective, while in inoperable patients, medical treatment is desired.</td>
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<td>Preventive measures including proper education, public awareness and effective policy measures to dispose the slaughtered animals are desired for control of infection.</td>
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<td>Andhra Pradesh, South India 6. Hemachander et al. [16]</td>
<td>Cross sectional patient based study; teaching hospital</td>
<td>Age preponderance not reported; out of 11 CE confirmed patients, 6 were females and 5 males</td>
<td>Out of 100 clinically suspected CE patients during 7 months period (November 2005 – May 2006), 11 (11%) were identified by ultrasonography, confirmed by surgery and examination of aspirated fluid.</td>
<td>Patients were not aware that the human infection can occur due to ingestion of contaminated material with the faeces of infected dogs.</td>
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<td>Chandigarh, North Indian states 7. Khurana et al. [17]</td>
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<td>Increasing trends in sero positivity in North Indian patients indicates the need to implement effective control measures.</td>
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<td>Chandigarh, North Indian states 8. Bakshi et al. [18]</td>
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<td>A total of 41 FNACs from 36 paediatric patients with liver space-occupying lesions over 5 years (1999-2004) showed, 1 (5.8%) patient confirmed as hydatid cyst.</td>
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<td>Kashmir Valley, North India 9. Fomda et al. [19]</td>
<td>Retrospective study; tertiary care hospital</td>
<td>Age range 12-68 years; male preponderance</td>
<td>During a period of 17 years (1984-2001), out of 5808 patients clinically suspected of CE, 705 (12.1%) were surgically confirmed.</td>
<td>The study suggests urgent need for control measures.</td>
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India are scanty [40]. Sero-epidemiological study revealed that out of 1429 asymptomatic persons residing in different districts of Kashmir (North India), 5% were seropositive. The significant factors associated with seropositivity were age <15 years, male gender, history of contact with dogs and rural residence [40]. The younger age group with seropositive response indicates that in endemic areas infection is acquired in childhood, supporting the worldwide, earlier well reported observation that infection is usually acquired in childhood, remaining asymptomatic for long period and in symptomatic subjects, symptoms may manifest in adult age depending upon the organ involved [2]. A retrospective analysis of 5808 patients in the age group 12 to 68 years, clinically suspected of CE, attending a referral hospital at Srinagar, Kashmir (North India) from 1984 to 2001 showed male preponderance, and out of these, 705 (12.1%) patients were surgically confirmed [19]. Another retrospective and prospective analysis of 117 patients attending a rural tertiary care hospital in Wardha, Maharashtra (West India) from 1996 to 2006 revealed that a majority of the patients presented in 21 to 30 years (third decade) age group [15]. In addition, an analysis of histopathology records of 91 patients from 1997 to 2004 and 26 surgically confirmed patients from 2005 to 2007 from the same hospital revealed that the commonly affected patients were in second and fourth decade [14]. In both the reports, females were predominately infected. It is suggested that in these areas, females are actively engaged in farming activities and livestock herding, thus likely to be more affected [14]. From Kolkata (East India), an analysis of five years cumulative data of hydatid lung disease also showed female preponderance with median age at presentation 33 years [13]. In contrast, a review of 118 cases attending various hospitals in central and southern epidemic zones of Andhra Pradesh (South India) from 2009 to 2011 showed male preponderance (72%), wherein a majority of the patients were in their third decade (25.4%) of life, and farmers (27.9%) were most affected [12]. It was suggested that in South India, males are usually more involved in farming, animal breeding and agricultural activities as compared to women. Most of the worldwide reports indicate female preponderance mainly due to their activities predisposing to infection [10]. Lack of awareness of the fact that CE can be caused by ingestion of food contaminated with the faeces of dogs may be one of the major factors leading to transmission of infection in endemic areas in India [16].

Molecular epidemiology

Strain variation in *E. granulosus* is well recognised and globally ten genotypes (G1-G10) have been documented [41]. Fingerprinting of 22 animal cyst isolates from eastern part of India using random amplification polymorphic DNA PCR technique has revealed genotypic variation in isolates from cattle, buffalo and sheep, while ITS1 based restriction fragment length polymorphism (RFLP) technique did not reveal any variation [42]. From western India (Maharashtra), molecular characterisation of 46 animal cyst isolates, based on cytochrome oxidase 1 (cox1) gene revealed G3 (buffalo strain) as the predominant type in 29, followed by G5 (cattle strain) in 9, G1 and G2 (sheep strain) in 6 and 2 isolates respectively [43]. From North India (Kashmir, Shimla, Punjab and Chandigarh), cox1 gene based genotyping has identified G1, G2 and G3 genotypes in animal isolates from different geographical areas [44]. Further, zoonotic potential of G1 (sheep strain) and G3 (buffalo strain) genotypes as predominant genotypes infecting humans was noted, and also, the first human CE case infected with G5 genotype (cattle strain) from an Asian country and presence of G6 genotype (camel strain) in India has been reported [45]. The profiling of prevalent genotypes may have important implications in understanding the epidemiology of this infection and for planning control strategies for hydatidosis.

**CLINICAL PRESENTATION**

The infection may remain asymptomatic or it may lead to varied symptoms depending upon the organ involved. A hospital based report showed that 14.2% of the infected persons were asymptomatic and were accidentally detected while investigating for some other pathology [13]. The clinical features of CE in symptomatic subjects are generally diverse and mimic other pathologies. The innumerable case reports from different regions in India have revealed liver [17], lung [13,20,21], brain [22], kidney [23], ovary [24], spleen [25], intra-abdominal cyst with indentation of the liver [26], peritoneum [27], bone [28], musculo-skeletal [29-32], subcutaneous [33-36], spinal cord [37], breast [38], and other organ involvement with associated complications [15,39]. Liver is the most common organ involved followed by lungs and other organs [15,17,19], and this observation is supported by innumerable reports worldwide [1,2,9,41]. A report from Wardha, Maharashtra, showed involvement of liver (75.2%), lung (14.5%), kidney and omentum (1.7%) in that order of frequency. The patients with lung hydatid (35.3%) presented to the hospital earlier, i.e. within a month of appearance of symptoms as compared to only 7.4% patients with abdominal hydatid; the majority (58.9%) of patients with abdominal hydatid presented within 6 months of appearance of symptoms [15]. The symptoms of liver hydatidosis are mainly pain in abdomen and lump in abdomen [14], while lung hydatidosis patients presented with cough as the commonest symptom (73.6%) followed by chest pain (54.7%). and the complications were lung abscess, pleural involvement, pneumonitis and fibrosis [13]. Unusually, lung hydatid cyst may also present with massive haemoptysis [39].

**Diagnosis**

The definitive diagnosis of CE can be achieved by the demonstration of scolices, hooklets or protoscolices in aspirated fluid by direct microscopic examination and/or stained smears. Fine-needle aspiration cytology (FNAC) based diagnosis has been commonly used for evaluation of space occupying lesions. In a series of 41 FNACs conducted in a tertiary care hospital in North India, one case of hydatid cyst was diagnosed, based on correlation of FNAC diagnosis with clinical, radiological, histological findings and patient follow-up [18]. In another retrospective analysis of 125 FNACs from the same area, 59 had a parasitic etiology, of which 8 were hydatid cysts [11]. However, fluid aspiration is not recommended usually, as there is risk of anaphylactic reaction. Moreover, its low sensitivity limits its usefulness, as microscopy has been reported positive in less than 50% samples [46]. Thus, the clinical diagnosis is usually substantiated by radiological and immunological techniques. Radiological techniques such as ultrasonography, computed tomography and magnetic resonance imaging have been found useful to categorize the
infection in various stages for management [47], however, radiological findings may mimic other pathologies, and thus antibody detection serves a useful adjunct to radiology for the diagnosis. The conventional serodiagnostic techniques, enzyme linked immunosorbent assay (ELISA), indirect fluorescent antibody (IFA), indirect haemagglutination (IHA) and latex agglutination (LA) tests have been applied for antibody detection with varying degree of sensitivity and specificity depending upon the type of antigen and technique used [48-51]. With the use of crude hydatid fluid antigen in highly sensitive technique(s), low specificity is reported. In a comparative evaluation of antibody detection by ELISA, IFA, IHA and Casoni’s test in 46 surgically confirmed patients, ELISA was positive in 100% patients irrespective of cyst localization, whereas IFA, IHA and Casoni’s test was positive in 90%, 76.2% and 71.4% liver hydatidosis and 81%, 59% and 50% of lung hydatidosis patients, respectively. The study concluded that ELISA is more sensitive and specific than the other techniques in diagnosing CE, especially lung infection [51]. Another retrospective analysis of data of 3290 clinically and/or radiologically CE suspected patients from a tertiary care hospital in north India showed that antibody response was positive in all the 296(100%) confirmed patients, whereas Casoni’s test was positive in only 90% liver hydatidosis, 50% spleen infected cases, 33.33% pulmonary and ophthalmic cases, and none of the patients with renal or brain cysts [17]. Antibodies cannot be detected in approximately 10% of patients with liver and 40% with lung cysts, and low antibody titres have often been found in patients with brain and ophthalmic cysts [52]. An evaluation of ELISA for detection of specific IgG and IgE indicated that IgG detection was better than IgE in a helminth-infested population as IgE was positive in significant number of non-hydatid patients [53]. This observation is in agreement with our recent study in an endemic area, indicating IgE WB percentage immunoreactivity significantly less than IgG WB in surgically confirmed cases and IgE immuno blot also yielded lower specificity [Personal communication].

The specific antibody detection in non-invasive samples such as urine and saliva is a desirable approach for the diagnosis of microbial infections. The sensitivity of ELISA for detection of specific antibodies in urine, serum and saliva was found 84%, 72% and 56%, respectively, and specificity was 72% in all the three types of samples collected from patients suspected clinically/radiologically of CE in a tertiary care hospital located in North India. Urine sample showed significantly better sensitivity as compared to saliva but no significant difference was observed as compared to serum samples [54]. It was extrapolated from the study that antibody detection in both urine and saliva samples (both non-invasive) without serum may yield 92% positivity which was much higher than with the use of only serum sample (72%). It was further suggested that 100% sensitivity can be achieved if antibody detection is carried out in all the three types of samples.

The detection of antigens excreted in body fluids is another approach to diagnose parasitic infections [55]. Circulating antigen may indicate recent and active infection and may also help in monitoring the efficacy of chemotherapy. A study from Puducherry (South India) showed urinary antigen in 43.8% samples from surgically confirmed hydatidosis patients. The specificity was 100% with respect to samples from other parasitic diseases, while the test was 8% false positive in samples from healthy subjects. Counter current immunoelectrophoresis (CIEP) and co-agglutination tests were evaluated for the detection of hydatid antigen in cyst fluids. Antigen detection by co-agglutination test was positive in 100% samples [46], while CIEP was found positive in 78.5% samples with 100% specificity [56]. Another study from North India reported that the sensitivity of ELISA for antigen detection in urine, serum and saliva was 52%, 40% and 24%, respectively, and specificity was 80%, 92.5% and 87.5%, respectively. Interestingly, there was no significant difference in hepatic and non-hepatic cysts or single and multiple cysts. It is thus suggested that urine sample may be used for antigen detection as an alternative or in addition to serum because of the non-invasive nature of sample collection and comparable sensitivity and specificity [57]. Further, detection of specific IgG1 and IgG4 antibodies in serum and urine samples had best correlation and it is suggested that urine sample could be a new approach for the diagnosis of CE [58].

The role of specific antibodies of different IgG subclasses and identification of highly dominant and specific immunoreactive antigen fractions have added new dimensions in the immunodiagnosis of parasitic diseases. Different immunogenic fractions have recognized different Ig classes depending upon the type of preparation of antigens and it is suggested that specific secretory IgA detection in hydatid fluid may serve useful purpose for the diagnosis [59]. The different fractions of diagnostic value have been reported from different geographical areas worldwide. The antibody detection to antigen 5 (EAg5) or to heat-stable lipoprotein in antigen B of E. granulosus has revealed different results in different reports as reviewed earlier [60,61]. The antibody to EAg5 is recognised in echinococcosis serum samples but is cross reactive with T. solium cysticercosis samples and thus may not serve useful purpose in T. solium infected endemic areas. Another echinococcus antigen with an apparent 8kDa molecular mass, not related to EAg5, was found 91% sensitive in western blot (WB) assay. This antigen was not cross reactive with cysticercosis samples but was genus specific [62]. A study conducted in a tertiary care hospital in North India revealed recognition of 8 and 116 kDa hydatid antigens in serum as a specific test for CE diagnosis [63]. Another study from Iran reported WB analysis of specific IgE and IgG subclass antibodies and found IgG4 and IgE to be the most significant antibodies for serodiagnosis in an Ag5 based immunoassay system [64]. The sensitivity of 60 kDa E. granulosus antigen (paramyosin) was 82% specific for IgG4 detection and only 33% for IgG antibody detection suggesting that IgG4 detection of E. granulosus paramyosin may be useful for the diagnosis of CE [65]. In addition, WB with purified antigens proved to be useful in the diagnosis and post-surgical monitoring of hydatidosis patients [66]. Our recent study showed that IgG4 antibody detection to 39 kDa E. granulosus antigenic fraction may prove to be useful for evaluation of post-treatment response [Personal Communication].

Immune response

Parasites have evolved multiple mechanisms to evade host immune response which favour survival and lead to chronic infection. In CE, chronic infection persists with detectable humoral
and cellular host immune response against the parasite. Although *E. granulosus* induces a strong humoral response in infected subjects, none of the Ig classes is associated with protection. Further, correlation of distinct IgG subclass antibody response with the host-parasite relationship and evading mechanisms is suggested [67]. *E. granulosus* releases molecules that directly modulate the host immune responses favouring a strong anti-inflammatory response resulting in successful establishment of infection and survival in the host. Studies suggest that IL-4/IL-10 impair the T helper cell-1 (Th1) protective response and allows the parasite to survive. Predominant T helper cell-2 (Th2) immune response in established CE is associated with increased susceptibility, while Th1 response is assumed to give protection. The Th2 cell dominant cytokine profile has been observed in CE patients in Puducherry, south India and it is suggested that further studies are required to evaluate the role of cytokine responses in eliciting pathogenicity of different strains prevalent in India [68]. Antigen B, *E. granulosus* tegumental antigen, *E. granulosus* elongation factor-1 B/8 (EgEF-1 B/8) and other parasite molecules elicit highly Th2 polarized response. All these antigens modulate the host immune response through intrinsic ability and by strengthening the generalized Th2 response. It is suggested that the further studies may throw light to recognise the extremely complex host-parasite interaction in CE [69]. Experimental animal models have been developed to study the drug efficacy and to evaluate potential protective antigens [70-71].

**Management**

Surgery remains the treatment of choice in hepatic and single cysts. PAIR (puncture, aspiration, injection and reaspiration) and percutaneous thermal ablation have been used for management of CE, though it is more successful for lesions in liver, yet it is not recommended for lung cysts [2,72,73]. PAIR is indicated for patients who cannot be operated and those who do not give consent for surgery. Albendazole therapy was found effective for patients who cannot be operated and those who do not give consent for surgery. Albendazole therapy was found effective in 61.5% of inoperable lung hydatid patients and in surgically treated patients when given concomitantly pre- and post-operatively [13].

**Control and prevention**

Control programmes for CE have been implemented in many endemic regions worldwide to reduce or eliminate CE as a public health problem. By 2002, Iceland, New Zealand, and Tasmania had already declared elimination of hydatid disease from their regions. Programmes for CE implemented in parts of South America, Europe and East Africa have yielded varying degrees of success and some were considered as failures [74]. To reduce the zoonotic risk and human cases, efforts are mainly directed towards treating domestic dogs and changes in husbandry practices. Treatment of dogs with praziquantel on a monthly basis and treating domestic dogs and changes in husbandry practices. Control and prevention and to ensure maintenance of the long-term continuation of the achieved control, especially in the advanced consolidation phase of the programs. In addition, vaccination of bovines against *E. granulosus* can protect sheep and goats [76]. Vaccination of sheep with EG95 vaccine reduces transmission to dogs and enables achievement of control earlier. It is suggested that combined control of CE with other zoonotic diseases should be evaluated for the cost-effectiveness, especially in inaccessible semi-nomadic populations, where CE is endemic [8]. Moreover, it is emphasized that broadening the agenda of CE control from the current focus on medical care to a focus on public health is essential for well-defined planning and execution of the prevention and control measures in India [73].

**CONCLUSION**

In conclusion, CE continues to be a significant public health problem of concern in India, as evident from the published reports from widely distributed geographical areas. The actual prevalence and incidence are difficult to interpret, as most of the reports are either cases or based on hospital records. The interpretation of prevalent age group may depend upon time and duration of infection and appearance of symptoms, while gender prevalence may depend upon geographical location and cultural practices in endemic areas. The clinical features are variable depending upon the organ involved. The early diagnosis and prompt treatment are few of the challenges. The knowledge of prevalent genotypes may help in planning the specific control programs. Awareness of mode of transmission of CE in general population in endemic areas, integrating the CE control strategies with other appropriate control programs, research focus on development and evaluation of newer control tools including immunological and parasite antigen detection studies for development of an efficient vaccine for dogs and sheep, development of clinically useful molecular and immunological methods for early diagnosis and treatment, and identification of newer effective drugs are necessary to provide a solid platform for improved control programs for CE.

**REFERENCES**


