TAJ June 2021; Volume 34 Number-1

ISSN 1019-8555
The Journal of
Teachers Association
RMC, Rajshahi

Original Article

Humoral Immune Responses to Amebic Liver Abscess

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Abstract

Background: Amebic liver abscess (ALA) is an ancient parasitic disease caused by E. histolytica described first by Hippocrates. It is endemic worldwide, mainly in tropic and subtropics countries. About 50 million true E. histolytica infections and approximately 100,000 deaths occur each year globally. In Bangladesh, exact incidences of amebic liver abscess cases are not estimated, but hospital reports indicate that it is endemic. Immunity and immune responses in acute and post infections of ALA are not well understood to date. However, the understanding of immunology is essential to know disease progression, recovery, morbidity, and mortality as well as diagnosis and newer prevention strategies like vaccine development. In this 15-month prospective and follow-up study, different antibody responses are estimated periodically.

Methods: About 90 amebic liver abscess patients diagnosed initially by ultra-sonogram confirmed followed by Real-Time PCR were selected for this study. All were admitted into Rajshahi Medical College Hospital, Bangladesh. Antibody responses against different antigens, which include Serum anti-lectin IgG, Salivary anti-CRD (carbohydrate recognition domain) Ig A, and Stool anti-CRD (carbohydrate recognition domain) IgA were estimated by ELISA periodically thrice, in acute stage after 06 and 09 months and between 12 and 15 months.

Results: Serum anti-lectin IgG in ALA persists remarkably high well up to 09 months in 98% cases, Secretory anti-CRD IgA was also determined from the saliva, and only 36(40%) show positive titer during first 06 months, and about 40% of ALA cases show high titer of anti-CRD IgA from stool samples in first six months of infection.

Conclusion: Only serum anti lectin Ig G showed significant high titer in 98% of cases in the acute stage and up to nine months of infection..

TAJ 2021; 34: No-1: 05-08

Introduction

Amebic liver abscess (ALA) is a parasitic disease caused by protozoa *E. histolytica*. It is a disease of the ancient period and was first described as a deadly disease by Hippocrates (460–377 BC.) In 1885, Alexdravisch Losch first *linked E. histolytica* as a cause of intestinal amebiasis[1], but *E. histolytica* was first recognized as an agent of amebic liver abscess in 1945 by famous Robert Koch.[2] Global burden of the amebic liver abscess has not been estimated precisely till date and can be considered as a neglected tropical disease. According to the WHO fact sheet, it is prevalent

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throughout the underdeveloped and developing nations of the tropics with up to 50 million *E. histolytica* infections, and approximately 100,000 deaths occur each year, mostly from liver abscesses or other complications.[3]

A few sporadic reports and hospital records claimed that amebic liver abscess is also endemic in Bangladesh, but its exact annual incidence has not been figured out. International center for diarrheal diseases research, Bangladesh (ICDDR,B) has done a few extensive studies, but mostly on intestinal amebiasis.[4]

From the patient record book of Rajshahi Medical College Hospital and few past studies, it can be speculated that approximately 120 -320 patients take admission each year.[5]

Immune response to amebic liver abscess is an important component related to the disease progression, recovery, morbidity, and mortality, as well as diagnosis and newer prevention strategies. Humoral immune response against *E. histolytica* infection is to some extent incomplete and not well understood. Different antibodies against different surface antigens of *E. histolytica* have been studied by different authors. The current study elucidated the height of the humoral immune response, both circulatory and secretory, at the time of infection and its post-infection period.

Methodology:

The study population included 90 amebic liver abscess patients who were admitted into Rajshahi Medical College Hospital, Bangladesh, during the period from July 2016 to June 2017, and 90 healthy volunteers of comparable age, sex, and ethnicity as controls.

The presence of a space-occupying lesion in the liver detected by ultrasound imaging substantiated initial clinical suspicion for ALA, which was later confirmed as amebic liver abscess through detection of small-subunit rRNA gene of *E. histolytica* in liver abscess aspirates by Real-Time PCR.

Serum anti-lectin IgG, Salivary anti-CRD (carbohydrate recognition domain) Ig A, and Stool anti-CRD IgA was estimated by ELISA at the time of enrolment, and follow-up samples of the same patients and controls were collected twice between 6-9 months and then between 12-15 months. Informed written consent was obtained from both patients and controls. The study was reviewed and approved by the Institutional Review Board (IRB) of Rajshahi Medical College and IRB of ICDDR,B.

Results:

In this study, the humoral immune response among study populations estimated for more than one year is noted by ELISA.

Serum anti-lectin IgG: Among 90 cases (N=90) at enrolment show, high titer of serum anti-lectin IgG gradually declined, at first follow-up 93% and in second follow-up only 36% specimens show OD value higher than cut off value. The result of serum anti-lectin IgG at acute stage compared with controls and first with acute stage and first follow-up value with second follow up value found statistically significant $P \leq 0.0001$.

Salivary anti-CRD IgA: Anti-CRD IgA from saliva was estimated among all patients (N=90) during admission, and 37 (41%) were positive and almost disappeared entirely during $^{\text{the first}}$ follow-up, and no control showed positive titer.

Stool anti-CRD IgA: Secretory anti-CRD (carbohydrate recognition domain) IgA from stool was measured, and 52(57%) only had high titer above cut-off value, $^{\text{first}}$ follow-up 27(30%) and $^{\text{second}}$ follow-up showed 24(27%) positive titer. Another interesting finding was among the controls, 09(10%) had high titer stool anti-CRD IgA.

Discussion:

Evaluation of antibody against *E. histolytica* and its persistence for 12 to 15 months in the amebic liver abscess is noted.

In the present work, a high titer of anti-lectin IgG was found in 98% of acute cases, and it persists at least for nine months, and very few patients (36%) had moderately high titer (OD>0.5 cut off) after 12 months on follow up. The inference that came from this study is serum anti-lectin IgG in ALA

persists remarkably well up to 6 -9 months (mean =225 days). All controls except one showed nonsignificant OD values for anti-lectin IgG (P-value ≥0.5), which is a statistically highly significant difference clearly demarcates the disease states and healthy state. This result contrasts a few past studies[7] which speculated that anti-amebic IgG persists for years.

Secretory anti-CRD IgA was also determined from the saliva from 90 ALA cases. Here, 36(40%) was positive, among 35 valid results of first follow up samples, none given positive or significant titer, but interestingly among 32 valid results from second follow up samples, 04(12%) had given positive results, and among 90 healthy controls 03(3.3%) had positive titer. It can be reasonably presumed that salivary secretory anti-CRD IgA is raised poorly during acute infection but does not sustain up to six months. Further, the results of control and second follow-up cases do not differ significantly. Raised secretory IgA refers to non-invasive amebiasis, but it cannot be correlated with invasive amebiasis [7,8]. However, the relative rise of titer of anti-CRD Ig A in second follow-up after disappearance on first follow-up may be a result of unnoticed invasion or entry by *E. histolytica.* Because most of the patients resided in an endemic zone for amebiasis and there is a good possibility that victims get protection at that period by anti-CRD IgA from a further ailment.

Anti-CRD IgA from a stool sample was estimated (N=70) and was found significant-high OD values for 40(57%) cases at enrollment, among the first follow up (N=36), 10(28%) was positive for anti-CRD IgA and in second follow up samples (N=30), 08(25%) shows positive titer and among the 90 healthy controls (N=90), 09 (10%) shows higher than normal titer. From these results, we can conclude that results of stool anti-CRD IgA can not demarcate efficiently healthy and disease states of ALA and possibly stool anti-CRD IgA is more pronounced in mucosal immune response like intestinal amebiasis. We can assume that a relatively similar pattern of high titer of stool anti-CRD IgA in our study population, including first, second follow up and healthy controls, may be due to the endemic existence of intestinal amebiasis as well as carrier status. However, it is known that secretory IgA from mucosa gives protection to some extent against amebic invasion in the intestine [9]. But the role of anti-CRD IgA cannot be ruled out in this event.

Conclusion:

Understanding of immunology on amebic liver abscess is an important part of the knowledge of *E. histolytica* induced invasive infection. The major interest of the present work was to study the immune responses, which included estimation of different antibody titers and duration of their presence in a human biological system. Our study revealed the development of high anti-*E. histolytica* IgG in serum in post amebic liver infection persists for about one year and can be a good diagnostic marker for amebic liver abscess, but protective immunity by this antibody is in question because of our study limitation. Secretory CRD IgA against carbohydrate recognition domain of *E. histolytica* lectin in both saliva and stool has given insignificant results related to amebic liver abscess but, there was no correlation between salivary and stool IgA response, suggesting independent inductive and effector sites in both compartments. These anti-CRD IgA responses are possibly more related to intestinal amebiasis and can be reasonably explained in this particular endemic area by restimulation of memory B lymphocytes at mucosal sites. For this reason, a more precise study regarding anti-CRD IgA may help to find out the role of its protective immunity against intestinal amebiasis.

Conflict of Interest:

The authors declare that they have no conflict of interest.

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