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IgG Anti-Body Titers among Patients with Amoebic Liver Abscess in Bangui, Central African Republic

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Abstract

Entamoeba histolytica (Eh) is a protozoan parasite that causes amoebiasis characterized by intestinal damage and amoebic liver development and is an important cause of hospitalization in low-middle income countries. The aims of this study were to determine the prevalence and the titer of IgG anti *Eh* in ALA (Amoebic Liver Abscess) patients' in Bangui according sex, age and other risk factors. This was a cross sectional study where data was collected between January 2018 and October 2020. The diagnosis of ALA was suspected based on clinical symptoms of; fever, abdominal pain (usually in the right hypochondrium or epigastrium) and clinical signs of hepatomegaly and/or tender liver with or without jaundice and abdominal ultra-sonography. ALA patients' IgG antibody titers were measured by Indirect Hemmaglutination Assay and Chi-square test was used. A total of 1249 ALA patients were included, among whom 570 (45.64%) were positive. Of these, 244 (42.08%) had titer 1:160 or less, 223 patients' (39.13%) had titer ranging from 1:320 to 1:640 and 103 patients (18.07%) had strong titer ranging from 1:1280 to 1:2560. The association between antibody titer results, age and sex was no significant (p = 1.0000). Our findings indicate a high prevalence of ALA and show no significant difference between the sex and age ALA patients.

Keywords

Amoebic Liver Abscess, Antibody, Sex Difference, Central African Republic

1. Introduction

Amoebic liver abscess (ALA) is caused by protozoan parasite *Entamoeba histolytica*, a common parasite infection in tropical countries. The estimated world-

wide prevalence of ALA is about 50 million infections per year with mortality ranging from 40,000 to 100,000. It is considered the third leading cause of death amongst the parasitic diseases [1]. Only in about 10% of the cases, the parasite evades from the gut leading to severe clinical disorders like hemorrhagic colitis or, in case of spread via blood stream, a destruction of the liver tissue, the amebic liver abscess. In contrast to amoebic colitis with similar or even higher infection rates in women, ALA mainly occurs in adult men [2] [3] [4]. Invasive amoebiasis is associated with the development of high anti immunoglobulin G (IgG) titers [5]. The expression of disease varies with geographical location. For example, in Egypt the predominant presentation is amoebic colitis, whereas in South Africa there is a high prevalence of ALA [6]. In Malaysia, it was reported that 44.1% of patients were positive for ALA [7] while 39% of patients had amoebiasis [8]. In Central Africa Republic (CAR), none of such work has been carried out in a patients' in Bangui and there has been no report on the prevalence of ALA in CAR compared to other countries in sub region. The present study was to determine the prevalence of ALA and the titer of IgG anti amoebic in patients suspected with ALA in Bangui. In addition, we also analyzed the results with respect to the sex age of the individuals and other risk factors.

2. Materials and Methods

2.1. Study Design

A cross sectional study was performed whereby all clinical or suspected ALA patients' who were admitted to the National Laboratory between January 2018 - October 2020 were included after obtaining an informed consent. Patients who were found to have positive bacterial culture of the pus aspirates from the liver and/or blood were excluded from the study. The patients' clinical data were collected from the hospital files. The diagnosis of ALA was suspected based on clinical symptoms of; fever, abdominal pain (usually in the right hypochondrium or epigastrium) clinical signs of hepatomegaly and/or tender liver with or without jaundice and abdominal ultra-sonography.

2.2. Data Collection

All clinical and sociodemographic data information of patients were recorded from the hospital files using questionnaire. Privacy and confidentiality was maintained by using patient's code during collection, compilation and analysis of data. Blood samples from each patient were separately collected into clean vacutainers® tubes (5 ml) and processed in the serology laboratory. The blood samples were centrifuged at 5000 × g for 5 minutes and the sera were separated and store in 4˚C to 8˚C or −20˚C until used.

2.3. Antibody Detection by IHA

Each serum sample was tested with Indirect Haemagglutination Assay (IHA) for antibody detection. The IHA was performed according to manufacturer's instruc-

tions (Fumouze diagnostic®): Each patient's serum samples were mixed with human group O erythrocytes sensitized with soluble, purified *E. histolytica* antigen in U-shaped micro titer wells. The specific antibodies present in the serum sample cross-link with the sensitized erythrocytes and the agglutinated erythrocytes will settle down in the well as carpet formation. For the qualitative test, the amoebiasis IHA reagent was diluted before the test with Tris buffer (pH 7. 2) in a 1: 10 ratio. Twenty-five microlitre of amoebiasis control serum positive was dispensed into well A1, 25 µl negative control serum into well A2 and beginning in with well A3, 25 µl of the samples were pipette into the remaining wells of the microtitration plate. A total of 50 µl diluted amoebiasis IHA reagent was added to each well containing the serum. The microtitre plate was placed on a shaker for 15 to 20 seconds, at 900 to 1100 rpm, then covered with polystyrene and subsequently incubated at room temperature without agitation for 2 hours. For the quantitative test, 50 µl Tris buffer (pH 7. 2) were dispensed into the first colunn (A1 through H1) and 50 µl of the buffer was dispensed into the remainimg wells except for A12. Then, 50 µl amoebiasis control serum positive was added to the buffer in well A1 and mixed well. Fifty microlitre of negative control serum was dispensed and diluted 1:40 in buffer solution pH7.2 into well A12. Then, 50 µl of samples to be tested were dispensed into wells B1 through H1 and mixed well with the buffer. Fifty microlitre was transferred from well 1 (A1 through H1) to well 2 (B2 through H2) and the serial dilutions were continued from rows A1 through A11. Finally, 25 µl from the last well was discarded. Subsequently, 25 µl amoebiasis IHA reagent was dispensed into the wells of rows 2 to 12, this corresponded to a starting dilution of 1:80. The microtitre plate was then placed on a shaker for 15 seconds then incubated without agitation for 2 hours. For the both qualitative and quantitative tests, the reaction was read by comparing the test samples to the controls using a reading mirror for microtitre plate. The test results were interpreted as positive whenever complete agglutination of the cells (carpet formation) were observed. A negative result was interpreted whenever the cells form sediment (button formation).

2.4. Statistical Analysis

Data was entered into Excel 2010 and analyzed by Epi info 3.5.3 from CDC Atlanta, 2011 version. Descriptive analysis was used for demographic data. Results were expressed as number and percentage for categorical variables whereas mean ± standard deviation (SD) and median were used for numerical variables. Fisher's Exact test and Mantel Haenszel Chi-square test were used to determine the correlation between concurrent sex and IHA with different antibody titer results and compared the odds ratio. A p-value < 0.05 were considered to statistically significant.

3. Results

3.1. Sociodemographic Characteristics and Antibody Titer Distribution

A total of 1249 patients with clinical or suspected ALA were included in the

study. Of these, 611 patients (48.91%) were males and 638 (51.09%) were females. The sex-ratio M/F was 0.95. Majority of patients were aged 16 to 67 years. The mean of age was 33.52 ± 12.90 years. A majority of patients from urban (84.86%) and alcoholic was (75.1%). The antibody response was found to be positive in 45.64% of patients. Among IHA positive titer group, 42.8% (244 patients) had titer 1:160 or less, 39.13% (223 patients) had titer ranging from 1:320 to 1:640 and 18.07% (103 patients) had strong titer ranging from 1:1280 to 1:2560. A total of 679 (54.36%) patients were IHA negative (Table 1).

3.2. Association between Antibody Titer and Sex

The analysis of total serum IgG within the same sex revealed that the antibody titer response did not differ significantly (p = 1.0000) between males and females' patients. However, among antibody titer positive group, 103 patients had very strong antibody response indicated by titer ranging from 1:1280 to 1:2560 and were not significantly (p = 0.09) more exposed (OR 2.19, 95% CI 0.94 to 5.1) by to amoebic liver abscess Table 2.

3.3. Association between Antibody Titer and Age Group

Patients were grouped into < 20 years or less, 21 to 30 years, 31 to 40 years, 41 to 50 years and >50 years for their age group. Majority of patients (46.74%) were from 2nd decade of their life. Similarly, only 9.7% of patients aged from 41 to 50

Table 1. Sociodemographic characteristic and distribution of antibody titer.

Characteristics		Number (%)
Average age (years) ± SD	34.66 ± 13.1	
sex-ratio M/F	0.95	
sex		
male		611 ((48.91)
female		638 (51.09)
Alcohol consumption		
Yes		938 (75.1)
NO		311 (24.9)
Habitat		
urban		1060 (84.86)
rural		189 (15.14)
Antibody titer group positive		570 (45.64)
1:160 or less		244 (42.80)
1:320 to 1:640		223 (39.13)
1:1280 to 1:2560		103 (18.07)
Antibody titer group negative		679 (54.36)

years had a strong antibody titer. The distribution and levels of serum IgG titer positive group specific to *Entamoeba histolytica* compared to age group were showed no significant association (p = 1.0000) Table 3.

3.4. Association between Antibody Titer and Other Risk Factors

ALA patients were divided into two groups; alcoholic and non-alcoholic, complicated and non-complicated in order to correlate the antibody titer. Out of 570 antibody titer positive, 428 were alcoholic and 86 were categorized as complicated ALA. Patients with these complications were categorized as complicated ALA and patients were categorized as alcoholic had a high antibody titer ranging from 1:1280 to 1:2560 respectively of 50% and 37.61% and found to have strong correlation respectively 3.15 [0.94 to 5.1] and 4.19 [0.94 to 5.1] $p < 0.05$ Table 4.

4. Discussion

The present study was carried out to determine for the first time the prevalence of amoebic liver abscess (ALA) in patients in Bangui, an endemic region of Central Africa Republic A detection of antibody titer among these groups may provide important information needed to support the diagnosis and treatment of ALA in the country. We reported 45.64% rates of amoebic liver abscess patients. As previously studies in Malaysia, the authors' reported 39% rates of amoebic liver abscess in patients [7]. The results from this study confirm that the expression of the disease varies with geographical location [6] [9] [10]. However, in developing countries where ALA is endemic, anti-amoebic drugs and antibiotics

Table 2. Relationship between antibody titer positive group and gender.

Characteristics	sex		OR [95% CI]	p-value
Antibody titer group positive	Male n (%)	Female n (%)		
1:160 or less	117 (47.95)	127 (52.05)	1.89 [1.12 to 3.6]	0.26
1:320 to 1:640	113 (50.67)	110 (49.33)	0.78 [0.33 to 1.53]	0.36
1:1280 to 1:2560	39 (37.86)	64 (62.14)	2.19 [0.94 to 5.1]	0.09

OR = Odds ratio, CI = Confidence Interval.

Table 3. Relationship between antibody titer positive group and age group.

Characteristics	age group (years)						
IHA titer	<20 n (%)	21 to 30 n (%)	31 to 40 n (%)	41 to 50 n (%)	>50 n (%)	Chi^2	p-value
1:160 or less	43 (17.62)	62 (25.40)	49 (20.1)	42 (17.21)	48 (19.67)	6.7	0.9
1:320 to 1:640	37 (16.6)	46 (20.62)	49 (21.97)	44 (19.73)	47 (21.08)	5.38	0.06
1:1280 to 1:2560	21(20.38)	21(20.38)	23 (22.34)	10 (9.7)	28 (27.20)	7.30	0.62

OR = Odds ratio, CI = Confidence Interval.

Table 4. Relationship between antibody titer positive group and other risk factor.

Characteristics	Alcohol		OR [95% CI]	p-value
Antibody titer group positive	no n (%)	yes n (%)		
1:160 or less	63 (44.4)	173 (40.42)	2.89 [1.12 to 3.6]	0.007
1:320 to 1:640	47 (33.1)	94 (21.96)	1.48 [0.33 to 1.53]	0.001
1:1280 to 1:2560	32 (22.5)	161 (37.61)	4.19 [0.94 to 5.1]	0.001
	Complicated ALA			
	no n (%)	yes n (%)		
1:160 or less	193 (39.9)	6 (6.97)	1.69 [1.12 to 3.6]	<0.05
1:320 to 1:640	110 (22.71)	37 (43.03)	0.65 [0.33 to 1.53]	<0.05
1:1280 to 1:2560	181 (37.39)	43 (50)	3.15 [0.94 to 5.1]	<0.05

OR = Odds ratio, CI = Confidence Interval.

may be used indiscriminately, where patients might have seen doctors in private medical centers or clinics for initial treatment. Some patients even had auto medication of antibiotics by buying the medication over the counter. Similarly, in this study, it was difficult to obtain an accurate treatment history and verification from all patients as to whether they have taken any medicine (medication name, dosage, and duration) prior to the admission. This could explain the high negative results of the antibody titer in our study. Our findings showed 18.07% of ALA patients had very strong response antibody titer. It has been previously observed that ALA patients have high anti-*E. histolytica* IgG antibody titers [11] [12] [13]. This would suggest that although subclinical invasion occurs continuously in women, it is controlled by their strong humoral IgG immune response, thereby suppressing the development of ALA at an early stage [14] [15]. Antibody *to E. histolytica* is known to persist for many months following a resolution of acute ALA. Thus the presence of an antibody may or may not signify acute infection. In this study, the cut of titer for positive antibody detection by IHA was1:320. Among IHA positive titer group, 42.8% (244 patients) had titer 1:160 or less, 39.13% (223 patients) had titer ranging from 1:320 to 1:640 and 18.07% (103 patients) had strong titer ranging from 1:1280 to 1:2560. This funding confirms the important role of serology test to support the diagnosis of ALA. It is well recognized that in *E. histolytica* infection, the antibody response varies with the individual patients and the type of infection. Furthermore, the higher antibody titer response against in ALA patients could be indicative of a more intense engagement of the immune system with the pathogen in the liver during invasive disease. However, these findings are in contrast to the work of Zeehaida *et al.* [9] where they reported 3.4% rates of ALA patients had very strong response antibody titer. Generally, these results showed that the distribution of the disease is geographically located. Also, these

results confirm the use of serological tests in the diagnosis of amoebic liver abscess in patients. Another finding of our study was that the antibody titer IgG immune response did not differ between male and female ALA patients. However, these results are in contrast to e previous studies where the authors reported that females had significantly higher anti-*E. histolytica* IgG [16]. However, due to the low number of ALA cases in women in this study, these results showed no significant difference. Furthermore, once ALA occurs, we confirm that ALA pathology proceeds equally in men and women. Also, the antibody IgG immune response had a strong complement activator, since the complement system is a major part of host innate immune defense against *E. histolytica* trophozoites, which are highly sensitive to complement-mediated lysis [15] [16]. In addition, the distribution and levels of serum IgG titer group compared to age group showed no significant association ($p = 1.0000$). However, these results showed that patients in their active age are important sociodemographic determinants for amoebic liver abscess and majority of this patient's was exposed in morbid complications of ALA, which was also noted in other studies [4] [17]. Alcoholic persons develop fatty liver and low cellular immunity which lead to deposition of large amount of iron that facilitates *E. histolytica* growth in the liver parenchyma leading to liver abscess has been revealed by Makkar *et al.* [18] and this correlation has been reinforced in our finding support this too. In addition to alcohol, factors such as living in rural areas, low socioeconomic condition and poor hygiene increased vulnerability to develop such pathology, which was also noted in other studies [4]. The serology test has become a valuable tool for diagnosis of ALA and detects specific circulating antibodies against the invasive of the parasite. It is good and useful test but the interpretation of the results are often difficult particulary in endemic areas where there are high background levels of seropositivity for amoebiasis. Thus, the interpretation of amoebic serology can be uncertain especially if the antibody titer is not high, what is a limit to this study.

5. Conclusion

Our findings indicate a high prevalence (45.64%) of amoebic liver diseases in the country. This high prevalence is alarming and indicates that ALA remains an issue of major concern in Central Africa Republic. In addition, our results showed no significant difference between the sex and age ALA patients. However, sex dependent differences in susceptibility and resistance to many infections in particular amoebiasis disease become unquestionably an increasing field of interest.

Authors' Contributions

WSN conceived, designed, conducted the experiments, analyzed the data and prepared the manuscript. **ERKL** read and approved the final manuscript. **JON** collected the sera and conducted the experiments.

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Institutional Review Board Statement

This study protocol received full approval from the local Ethics Committee of Health Science Faculty of University of Bangui and was conducted in compliance with the declaration of Helsinki. Approval reference number 22/UB/FACSS/CES/20.

Data Availability Statement

Data is contained within the article.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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