

ANTICORPS IGg ANTI-ENTAMOEBIA HISTOLYTICA CHEZ DES PATIENTS PRÉSENTANT UN ABCÈS AMIBIEN DU FOIE À BANGUI, RÉPUBLIQUE CENTRAFRICAINE

NAMBEI WS¹, GBANGBANGAI E², NDOMACRAH AB¹, KAMADJI L¹

RESUME

Objectif : l'objectif de cette étude était de déterminer la prévalence et le titre en anticorps anti-Entamoeba histolytica chez des patients suspects d'abcès amibien du foie et d'analyser les résultats en rapport avec le sexe et l'âge.

Méthodologie : Nous avons conduit une étude transversale analytique. Les données ont été collectées de janvier 2007 à octobre 2010 chez des patients présentant sur le plan clinique les signes suivants : fièvre, douleur abdominale incluant douleur de l'hypochondre droit, l'hépatomégalie. La méthode d'hémagglutination indirecte avait été utilisée pour le dosage des titres en anticorps. Le test de X2 avait été utilisé pour déterminer la corrélation entre les titres en anticorps, le sexe et l'âge.

Résultats : Au total, sur 1049 patients inclus, 470 (44,80%) étaient positifs. Cent quatre vingt-dix neuf patients (42,34%) avaient un titre inférieur ou égal à 1/160ème, 178 patients (38,88%) avaient un titre variant entre 1/320ème et 1/640ème et 93 patients (19,78%) avaient un fort titre variant entre 1/1280ème et 1/2560ème. Il n'existait aucune association significative ($p = 1,0000$) entre les titres en anticorps et le sexe.

Conclusion : Nos résultats avaient montré une forte proportion des patients avec abcès amibien du foie. Il n'existait pas de corrélation entre le sexe, l'âge et l'abcès amibien du foie. cette forte prévalence est alarmante et indique que l'abcès amibien du foie devient un sujet de préoccupation majeur dans le pays.

Mots-clés : Entamoeba histolytica, abcès amibien du foie, anticorps, sexe, Centrafrique.

ABSTRACT

ANTI-ENTAMOEBIA HISTOLYTICA IGg ANTI-BODY TITERS AMONG PATIENTS WITH AMOEBIC LIVER ABSCESS IN BANGUI, CENTRAL AFRICAN REPUBLIC.

Objectives: The aims of this study were to determine the prevalence and the titer of IgG anti amoebic in ALA patients' in Bangui and to analyze the results with respect to sex and age.

Materials and Methods: This was a cross sectional study where data was collected between January 2007 and October 2010. The diagnosis of ALA was established 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 ultrasound. ALA patients' IgG antibody titers were measured by Indirect Hemmagglutination Assay and Chi-square test were used.

Results: A total of 1049 Liver Abscess patients were included, among whom 470 (44.80 %) were ALA positive. Of these, 199 (42.34 %) had titer 1:160 or less, 178 patients' (38.88 %) had titer ranging from 1:320 to 1:640 and 93 patients (19.78 %) had strong titer ranging from 1:1280 to 1:2560. The association between antibody titer results and sex was no significant ($p = 1.0000$).

Conclusion: Our findings indicate a high prevalence of ALA and showed no significant difference between the sex ALA patients. This high prevalence is alarming and indicates that ALA remains an issue of major concern in Central Africa Republic

Keywords: Entamoeba histolytica, amoebic liver abscess, antibody, sex difference, Central Africa Republic.

INTRODUCTION

Amoebic liver abscess (ALA) is caused by protozoan parasite Entamoeba histolytica, a common parasite infection in tropical countries. The estimated worldwide prevalence of ALA is about 50 million infections per year with mortality ranging from 40,000 to 1, 000, 00. 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 amoebic 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 pre-

(1) Department of Sero-Immunology, Molecular Biology and Biochemistry, National Laboratory of Public health, Bangui, Central African Republic BP 1402 Bangui RCA.

(2) Department of Infectious Diseases, Army Health Care Service.

Auteur correspondant : wilfrid.nambei@gmail.com/ tél : 236 75509075

dominant 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 and age of the individuals.

MATERIALS AND METHODS

Type, population and period of study.

A cross sectional study was performed whereby all clinical or suspected Liver Abscess (LA) patients' who were admitted to the four (4) Hospitals University Centers in Bangui (HUCB) whose the blood samples were collected and sent to the National Laboratory between January 2007 - October 2010. All the patients' 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), abdominal tenderness, clinical signs of hepatomegaly and/or tender liver with or without jaundice and abdominal ultra sonography.

Data collection.

All clinical and sociodemographic information of patients' were recorded 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 5,000 x g for 5 minutes and the sera were separated and store in 4 to 80 C or -200 C until used.

Ethical Consideration.

Informed written consent was obtained from each patient' and the study protocol was approved by the Scientific and Ethical committee of Health Science Faculty of University of Bangui No 18/UB/FACSS/CSCVPER/07.

Laboratory Analysis

Antibody detection by IHA.

Each serum sample was tested with Indirect Haemagglutination Assay (IHA) for antibody detection

(positive threshold > 1: 160). All the patients IHA positive were seen again for a definitive confirmation of ALA one month later.

The IHA was performed according to manufacturer's instructions (Fumouze Diagnostic®). Each patient's serum samples was 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 column (A1 through H1) and 50 µl of the buffer was dispensed into the remaining 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).

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.

RESULTS.

Sociodemographic characteristics and antibody titer distribution.

A total of 1049 patients with clinical or suspected ALA were included in the study. Of these, 511 patients (48.71 %) were males and 538 (51.29 %) 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. The antibody response was found to be positive in 44.80% of patients (470/1049). Among IHA positive titer group, 42.34% (199 patients) had titer 1:160 or less, 38.88% (178 patients) had titer ranging from 1:320 to 1:640 and 19.78% (93 patients) had strong titer ranging from 1:1280 to 1:2560. A total of 579 (55.20 %) patients were IHA negative (table 1.)

Table 1: Sociodemographic characteristic and distribution of antibody titer/ Caractéristiques sociodémographiques et distribution des titres en anticorps.

Characteristic	Number (%)
Average age (years) ±SD	33.52±12.90
sex-ratio M/F	0.95
sex	
male	511 (48.71)
female	538 (51.29)
Antibody titer group positive	470 (44.80)
1:160 or less	199 (42.34)
1:320 to 1:640	178 (38.88)
1:1280 to 1:2560	93 (19.78)
Antibody titer group negative	579 (55.20)

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, 93 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

2.2, 95%CI 0.94-5.09) by to amoebic liver abscess table 2.

Table 2: Relationship between antibody titer positive group and gender/Relation entre le genre et les titres positifs en anticorps.

Characteristics	sex		OR [95%CI]	p-value
	Male	Female		
Antibody titer group positive	n (%)	n (%)		
1:160 or less	97 (48.74)	102 (51.26)	1.99 [1.11 to 3.57]	0.270
1:320 to 1:640	90 (50.56)	88 (49.44)	0.72 [0.40 to 1.33]	0.363
1:1280 to 1:2560	42 (45.16)	51 (54.84)	2.2 [0.94 to 5.09]	0.09

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 (44.68%) were from 2nd decade of their life. 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.

Table 3: Relationship between antibody titer positive group and age group/Relation entre les groupes d'âge et les titres positifs en anticorps.

Characteristics	age groupe (years)					Chi-square	p-value
	<20	21 to 30	31 to 40	41 to 50	>50		
IHA titer	n (%)	n (%)	n (%)	n (%)	n (%)		
1:160 or less	34 (17.09)	53 (26.63)	40 (20.10)	33 (16.58)	39 (19.60)	6.89	0.878
1:320 to 1:640	28 (15.73)	37 (20.79)	40 (22.47)	35 (19.66)	38 (21.35)	4.38	0.067
1:1280 to 1:2560	19 (20.43)	19 (20.43)	21 (22.58)	8 (8.60)	26 (27.96)	7.32	0.625

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 36.31% rates of amoebic liver abscess patients. As previously studies in Malaysia, the authors' reported 44.1% 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]. The serology tests have an important role in supporting the diagnosis of ALA. It is well recognized that in E. histolytica

infection. The antibody response varies with the individual patient and type of ALA. It is greatest in ALA, less in intestinal amoebiasis, and least in asymptomatic cyst passers [10]. However, the drawbacks of antibody detectable are that the antibody may not be detectable in the early phase of the infection. On the other explanation, antibodies to *E. histolytica* are known to persist longtime following a resolution of acute ALA. Thus the presence of an antibody may or may not signify acute infection, hence reducing the value of serological diagnostic test among patients with acute disease [10]. Moreover, in developing countries where ALA is endemic, anti-amoebic drugs and antibiotics 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. Associated with the other causes of the liver abscess (Pyogenic liver abscess, unclassified) this reason could partially explain the high negative results of the antibody titer in our study. Our findings showed 18.84% 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]. 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 [15]. 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* IgGt [13]. 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 comple-

ment-mediated lysis [14, 15]. 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 [16].

CONCLUSION:

Our findings indicate a high prevalence (44.80 %) 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 ALA patients. However, sex dependent differences in susceptibility and resistance to many infections in particular amebiasis disease become unquestionably an increasing field of interest.

Competing interests:

The authors declare that they have no competing interest.

Authors' contributions:

WSN conceived, designed, conducted the experiments, analyzed the data and prepared the manuscript. EG and NAB read and approved the final manuscript. LK collected the sera and conducted the experiments.

Acknowledgements:

This work was supported by the Directorate of National Laboratory of Public Health (NLPH). The authors wish to thank the staff of the NLPH for their help and support during the study.

REFERENCES

1. World Health Organisation. Amoebiasis. *Wkly Epidemiol Rec* 1997, 72: 97-99.
2. Haque R, Mollah NU, Ali IKM et al. Diagnosis of amoebic liver abscess and intestinal infection with the TechLab Entamoeba histolytica II antigen detection and antibody test. *J Clin Microbiol* 2000, 38(9): 3235-3239.
3. Haque R, Neville LM, Hahn P, Petri WA. Rapid diagnosis of Entamoeba histolytica infection by using Entamoeba and Entamoeba histolytica stool antigen detection kits. *J Clin Microbiol* 1995, 33(10): 2558-2561.
4. Blesmann J, Van Linh P, Nu PA et al. Epidemiology of amoebiasis in a region of high incidence of amoebic liver abscess in central vietnam. *Am J Trop Med Hyg* 2002, 66(5): 578-583.
5. Lotter H, Jackson TF, Tannich E. Evaluation of

three serological tests for the detection of amoebic antibodies applied to sera of patients from an area endemic for amoebiasis. *Trop Med Parasitol* 1995, 46(3):180-182.

6. Ravdin JI, Stauffer WM. *Entamoeba histolytica* (amoebiasis). In Mandell, Douglas and Bennetts principles and practices of infectious diseases. 2005, pp.3097-3110. 6th ed. Churchill Livingstone ; Philadelphia.

7. Goh KL, Wong NW, Paramsothy M et al. Liver abscess in the tropics: experience in the university Hospital, Kuala Lumpur. *Pst Grad Med J* 1987, 63: 551-554.

8. Jamaiah I, Shekhar KC. Amoebiasis: a 10 years retrospective study at the University Hospital, Kuala Lumpur. *Med J of Malaysia* 1999, 54(3): 296-302.

9. Zeehaida M, Wan Nor Amilah WAW, Amry AR et al. a study on the usefulness of Techlab *Entamoeba histolytica* II antigen detection ELISA in the diagnosis of amoebic liver abscess (ALA) at Hospiatl university Sains Malaysia (HUSM), Kelantan, Malaysia. *Trop Biomed*. 2008, 25(3): 209-216.

10. Hira PR, Iqbal J, Al-Ali F et al. Invasive amoebiasis : challenges in diagnosis in a non-endemic country (Kuwait). *Am J Trop Med Hyg* 2001, 65(4): 341-345.

11. Hock GM, Foon KL Chuen HI, Choo NG, Singh M. determination of antiamoebic IgG, IgM, IgA and IgG subclasses in sera from patient's amoebiasis by enzyme linked immunosorbent assay. *Southeast Asian J Trop Med Public Health* 1989, 20(3): 407-414.

12. Kaur U, Sharma AK, Vohra H. Distribution of *Entamoeba histolytica* Gal/galNAc lectin specific antibody response in an endemic area. *Scand J Immunol* 2004, 60(5): 524-528.

13. Bernin H, Marggraff C, Jacobs T et al. Immune markers characteristics for asymptotically infected and diseased *Entamoeba histolytica* individuals and their relation to sex. *BMC Infect Dis*. 2004, 14: 621.

14. Reed SL, Gigli L. lysis of complement-sensitive *Entamoeba histolytica* by activated terminal complement components. Initiation of complement activation by extracellular neutral cysteine proteinase. *J Clin Invest* 1990, 86(6): 1815-1822.

15. Snow M, Chen M, Guo J et al. Differences in complement-mediated killing of *Entamoeba histolytica* between men and women-an-explanation for the increased susceptibility of men to invasive amoebiasis. *Am J Trop Med Hyg* 2008, 78(6): 922-923.

16. Blesman J, Van LP, Tanich E. epidemiology of amoebiasis in a region of high incidence of amoebic liver anscess in Central Vietnam. *Am J Trop Med Hyg* 2002, 66(5): 578-583.