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Severity of *Entamoeba histolytica* infection associated with high CRP- level among Sudanese patients admitted with acute diarrhea.

AbdelazimShakir*[1], Tayseer E. Mohamed[1]; Mohamed B. Saad[2]
[1]*Parasitology Department, FMLS, Sudan University of Science and Technology.*
[2]*Parasitology Department, FMLS, Omdurman Ahlia University. Sudan.*

Abstract: *This study was conducted in conjunction with increase rate of acute diarrhea in Khartoum state during 2016, admitted patients infected with only E. histolytica appears with severe sign and symptoms, like 3-4 times diarrhea per day, low grade of fever, vomiting and elevated C-reactive protein (CRP) level,which were observed among more than 60% ofadmitted patients with acute amoebic dysentery. Beside that most of patients were given a combination drug which is more effective than metronidazole alone. Our report indicates that new invasive or mutant strains were related to the severity of acute amoebiaisis. Thus,large scale molecular study for amoebiasistogether withantibiotic susceptibility test is the method of choice to identifyingthese virulence strains and to determine the effective antibiotics for patient care.*
Keywords: Entamoeba histolytica, acute diarrhea,CRP.

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I. Introduction

Gastrointestinal infections are major causes of morbidity and mortality throughout the world and particularly in developing countries (1). The World Health Organization (WHO) ranks diarrheal disease as the second most common cause of morbidity and mortality worldwide(2). *Entamoeba histolytica*, it's one of the most three important diarrheacausing protozoa. (3),several members of the genus *Entamoeba* infect humans. Among these only *E. histolytica*is considered pathogenic and the disease it causes is called amebiasis or amebic dysentery, while other non-pathogenic amoeba with no symptoms of invasion and no treatment needed. Humans are the only host of *E. histolytica* and there are no zoonotic reservoirs. *E. dispar*is morphologically identical to *E. histolytica*and the two were previously considered to be the same species. However, genetic and biochemical data clearly indicate that the nonpathogenic *E. dispar*is a distinct species. The two species are found throughout the world, but like many other intestinal protozoa, they are more common in tropical countries or other areas with poor sanitary conditions (4). High rates of amebiasis occur in the Indian subcontinent, the Far East, western and southern Africa, and parts of South and Central America. In the United States and Europe amebiasis is found primarily in immigrants from endemic areas. The actual incidence of ameobiasis throughout the world, especially in the temperate zone, remains unknown (5).Surveys indicates that the incidence of infection varies from 0.2-50% and is directly correlated with sanitary conditions (3). In travelers, *Entamoeba histolytica*and *Giardia lamblia* are the most frequent causes of intestinal protozoan infection (6).*E. histolytica* is estimated to infect about 50 million people worldwide. Previously, It is estimated that up to 10% of the world's population may be infected with either *E. histolytica*or *E. dispar*(or both) (4). Mammals such as dogs and cats can become infected transiently, but are not thought to contribute significantly to transmission (7). The recognition of *E.dispar* as a separate non-pathogenic species meant that the results of all previous prevalence studies based on microscopy were not reliable. It was realized that *E. dispar*gave rise to about 90% of the 500 million new *amoeba* infections originally estimated to occur each year (8). It also became evident that, at most, only one in four real *E. histolytica*infections progresses to disease (9). For over 100 years, microscopy remained the only method for diagnosing intestinal *Entamoeba* infection, and even though it cannot differentiate between *E. histolytica* and *E. dispar*, it is still the technique of choice in many parasitology laboratories worldwide. In light of our present knowledge, microscopy must be considered as a screening method for the *E. histolytica*/*E. dispar* complex and not as a technique to confirm the diagnosis of *E. histolytica*. (10). Differential detection of two morphologically indistinguishable protozoan parasites *Entamoeba histolytica* and *E. dispar* has a great clinical and epidemiological importance.Difficulty In the diagnosis of amoebiasis is due to the presence of similar amoeba that can be misdiagnosed such as *Entamoeba dispar*and other noninvasive amoebae (11). Our objective in this study to focus on severity of cases reported of severe amoebiasis with moderate elevation in CRP level mainly in children less than 10 years and teenage patients admitted to hospital with acute diarrhea. C-reactive protein (CRP) is one of the classic acute phase proteins. (12). It was thought that CRP might be a pathogenic

secretion as it was elevated (100-1000 fold) in the people with a variety of illness such as infections, trauma, surgery, burn and malignant diseases. It's thought to bind to phosphocoline thus initiating recognition and phagocytosis of damaged cells (13). Measuring and charting of CRP value can prove usefulness in determining disease progress or the effectiveness of treatment (14). In children living in malaria endemic region elevated CRP level concentration are common, its mean level(7-8 mg/dl) (15)**.** Plasma CRP level can be valuable for identification of diseases and follow up aftertreatment of some diseases like kalazar(16)**.**

II. Material And Methods

2.1. Study design: aninconsecutive case series study was done on a selected group of diarrheic patients admitted to hospital in period between Apr. to Dec, 2016.

2.2. Subjects: A total of 50 patients were selected in this casereport. They were admitted to hospitals with acute form of diarrhea. All stool samples were diagnosedas Amoebic dysentery by direct fecal exam after formal ether technique (FECT). Ethical clearance of this report was obtained from Research Committee, College of Medical Laboratory Science at Sudan University of Science and Technology. The objective of the report was explained at the beginning to all patients under the study and a written consent was obtained from each participant. Also, a questionnaire was designed to collect data from the patients.

2.3. Sample: 5ml blood was collected from 50 dysenteric patients admitted at different hospitals in Khartoum state, Sudan, with complains of gastrointestinal discomfort, diarrhea associated with blood or mucus, low grade of fever and vomiting. No any other parasitic infections reported in case study group.

2.4. Measurements of CRP level: The CRP level was measured using latex turbidity. The CRP conc mg/L were obtained as differences from samplesA2-A1,divided by calibrator differences and multiplied by calibrator concentration. Results equal to 6 mg/L or higher consider as CRP positive.

2.5. Statistic evaluation: Statistical analysis was performed using SPSS version 16 (Statistical Package for Social Sciences).

III. Result

50 stool and blood samples were collected from admitted patients of 27 males and 23 females with clinical symptoms of gastroenteritis, diagnosed by microscopy after FECT as amoebic dysentery. No other parasitic or bacterial infections were reported. The mean average age of patients was (25.5±.02 years). (7)14% are children under 10 years old. Pathological Pictures of amoebic dysentery occurs in 73% of specimens (table1). As a diagnostic criteria under microscopy for the 50 fecal samples, trophozoite stage appear in 31specimens (61%), while cystic stage occur in 19 (19%) and 10(20%) for both stages. Highest CRP- level was detected at age group (1-10) years. [7.03]mg/L,Low CRP level observed at age group (21-30) years. [5.10]. mg/L. Mean CRP level was 6.06 mg/L. There is no significant difference in CRP level between age group. P. Value equal 0.52[Fig.1].

Table (1):Details of stool pictures in patients with acute amoebiasis:

Stool picture/Severity in grade	4+	3+	2+	1+	N	Total
Mucus	7	2	12	15	14	50
Pus cell	11	12	10	10	7	50
Red blood cell	16	5	4	12	13	50
Motile bacteria	14	2	4	10	20	50
Severity %	24%	10.5%	15%	23.5%	27%	100%

Table (2): C-reactive protein level among study group:

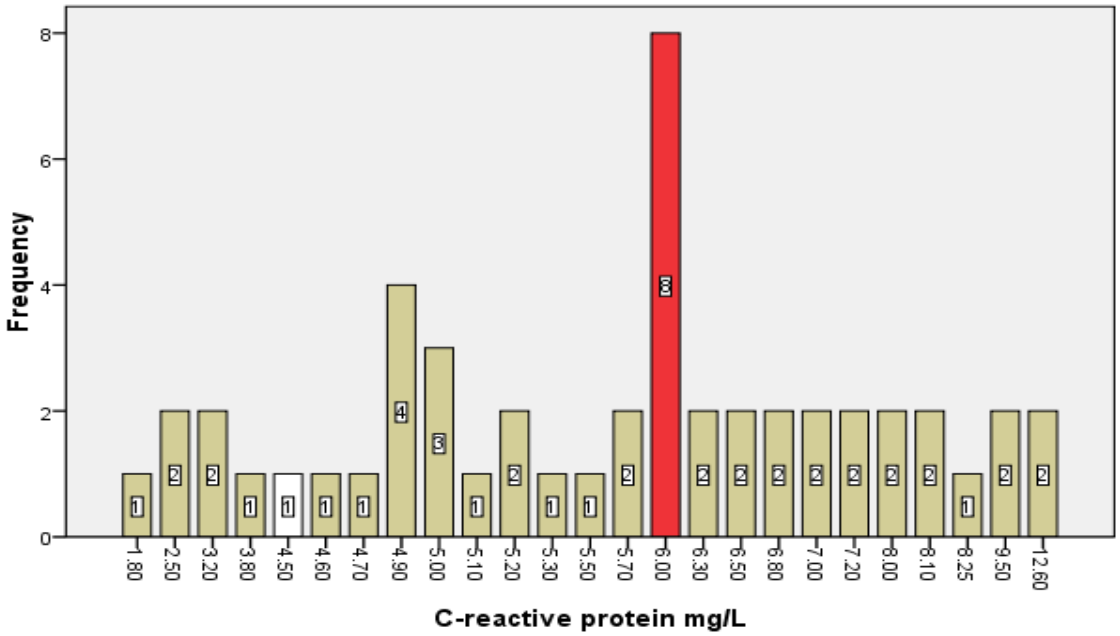
		sex		
		male	female	Total
CRP	**Positive**	15	12	27
	Negative	12	11	23
Total		27	23	50

Table (3):Mean CRP level according to age group infected with *E. histolytica*:

Quantitative CRP Concentration								
		Age group						
	Age	> 10	10 - 20	21 - 30	31 - 40	41 - 50	51 - 60	**Total**
CRP	**Positive**	5	4	8	5	4	1	27
	Negative	2	1	11	6	2	1	23
Total		7	5	19	11	6	2	50

Quantitative CRP Concentration								
	Age group							
	Age	> 10	10 - 20	21 - 30	31 - 40	41 - 50	51 - 60	**Total**
CRP	**Positive**	5	4	8	5	4	1	27
	Negative	2	1	11	6	2	1	23
Mean mg/L		7.03	6.61	5.10	5.30	6.55	5.15	6.06

Fig (1): Concentration of CRP among case group infected with *E. histolytica*:



IV. Discussion:

Our data reports confirm that, all admitted patients have an increased severity of diarrheal disease.The rates of diarrhea associated with high CRP werefoundin (27/50) 54% of admitted Sudanese patients infected with E. *histolytica* inKhartoum state, most of them are school children and teenage patients. Besides that, amoebic dysentery can spread rapidly and become serious infection when it finds favorable environmental and host factors' conditions, especially in low income countries. This result was similar to that of Hiro Mohammad Obaid, (17). These factors may leads to increase the prevalenceof the disease among different parts of our country, so anurgent action with effective and more powerful preventive measuresmust be taken.

V. Conclusion

Severity of *E. histolytica*infections in the reported cases it's not due to individual factors like weakness, nutrition state or low immunity, butserious clinical symptomswere observed in admitted patients caused byinvasive strains of *E. histolytica*. Molecular diagnosis for isolated strains of *E. histolytica*must be performedtogether withantibiotic susceptibility testto determine the genetic variation if present and to detect drug resistant mainly metronidazole for successfulpatienttreatment.Improve human hygiene and environmental sanitation to minimize the transmission of the disease.

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