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ORIGINAL ARTICLE

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Intestinal parasitic infection among Egyptian children with chronic liver diseases

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Abstract Patients with chronic liver diseases (CLD) are often highly susceptible to parasitic infection due to a depressed immune system. The objective of this study was to detect the most commonly intestinal parasites found among Egyptian children with CLD. The present study was conducted on 50 children with CLD of different etiology (25 were having different intestinal symptoms, 25 without intestinal symptoms) and 50 non-CLD children with gastrointestinal complaints served as controls. All cases were subjected to stool examination and investigated by liver function tests. Also, anthropometric measurements were taken for all children including weight and height. It was found that the most commonly intestinal protozoa identified in the patients with CLD in order of frequency were: *Entamoeba histolytica/Entamoeba dispar* (16 %), *Giardia lamblia* (14 %), *Blastocystis hominis* (14 %), *Cryptosporidium parvum* (10 %), *E. histolytica* and *G. lamblia* (2 %), *E. histolytica* and *B. hominis* (2 %), *G. lamblia* and *B. hominis* (2 %), *B. hominis* and *Entamoeba coli* (2 %), *Microsporidium* (2 %) and no cases were found infected with *Strongyloides stercoralis*. As compared to the controls, the observed incidence of these organisms in CLD patients was significantly higher ($p < 0.045$) as regards stool examination by unstained techniques while, there was no significant difference between both groups as regards stool examination by stained techniques ($p < 0.478$). In addition, this study showed that the weight and height of studied patients were affected by parasitic infection while, there was no significant correlation between parasitic infection and liver function tests. In conclusion, chronic liver diseases affect the immunity of the patients as shown in significant increase in the incidence of intestinal parasites in cases compared to controls.

Keywords Chronic liver diseases · Intestinal parasites · Stool examination

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Introduction

Chronic liver diseases (CLD) and cirrhosis are the most important health problems according to the current gastroenterology literature (Kirmaz et al. 2004). Hyperglobulinemia and depressed cell mediated immunity are common in various forms of CLD where the immunological derangements run parallel to the extent of the liver damage (Unger et al. 1986). Hepatic cirrhosis is characterized by altered handling of antigens due to depression of both humoral and cell mediated immunity (Kunkel et al. 1993).

Enteric protozoa and sporozoa have emerged as important opportunistic parasites and can cause fatal infections in immunocompromised patients (Tuli et al. 2010). Patients with CLD can be considered immunocompromised and they are susceptible to a wide spectrum of parasitic infection such as *Giardia lamblia* (*G. lamblia*), *Entamoeba histolytica* (*E. histolytica*), *Blastocystis hominis* (*B. hominis*), *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Isospora belli* and *Microsporidia* (Hegab et al.

2003). These parasites are frequently transmitted by unhygienic habits such as direct transfer of cysts from anal region to mouth, or eating and drinking of contaminated food and water.

In patients with CLD, successful recognition and management of parasitic infection result in avoidance of complications as electrolyte disturbance, dehydration and progression into a state of hepatic encephalopathy (Hegab et al. 2003). So this study was designed to detect the common intestinal parasites among Egyptian children with CLD.

Subjects and methods

The present study was conducted on 50 children; group I (GI) (31 males and 19 females with the mean age 8 ± 5.1 years) with clinical or biochemical evidence of CLD recruited from Hepatology Clinic, Ain-Shams University during the period from September 2011 to May 2012. They were classified into two subgroups according to the presence (GIa) or absence (GIb) of intestinal symptoms. In addition, control group (GII) comprised of 50 non-CLD children with gastrointestinal complaints who were age and sex matched with GI. All cases were subjected to detailed history, clinical examination and investigated by liver function tests; alanine transferase (ALT) and aspartate transferase (AST). Also, anthropometric measurements were taken for all children at the initial visit including weight and height.

At least three subsequent stool samples were obtained from each patient for detecting parasites ova, protozoal cysts and/or trophozoites. Stool samples were examined directly by wet mount preparation using saline and iodine methods, and then reexamined after concentrating them in formalin-ether (WHO 1991). In addition, staining with acid-fast trichrome (AFT) stain (Ignatius et al. 1997) and modified Ziehl-Neelsen (ZN) stain (Henriksen and Pohlenz 1981) was prepared for coccidial identification. In addition, Harada Mori culture was made for *Strongyloides stercoralis* (Denham and Suswillo 1995). The study was approved by Research Ethics Committee, Faculty of Medicine, Ain-Shams University, and informed consent was obtained from the children's parents.

Statistical analysis

The positive findings were expressed as a percentage, and the statistical analysis was carried out using Chi square test (χ^2). Probability (p value) <0.05 was considered statistically significant.

Results

Results are shown in Tables 1, 2, 3, 4, 5, 6, 7, 8.

Discussion

Chronic liver diseases in children are relatively common disorders with minimal symptoms but long-term risk of significant morbidity and mortality particularly in developing countries (Suchy 2007). The term "chronic liver disease" encompasses a large number of conditions having different etiology and existing on a continuum between hepatitis infection and cirrhosis (Abenavoli et al. 2007). In the present study, the etiology of CLD were biliary atresia in 12 (24 %), chronic HBV in 2 (4 %), autoimmune hepatitis in 11 (22 %), chronic HCV in 5 (10 %), metabolic causes in 7 (14 %) [Glycogen storage diseases in 3 cases, Wilson's disease in 3 cases and Galactosemia in one case], Budd-Chiari syndrome in 4 (8 %) and other different multiple causes in 9 (18 %) of them (drug induced hepatitis in one case, hydatid disease in one case, choledochal cyst in one case, congenital hepatic fibrosis in 2 cases, sclerosing cholangitis in 2 cases, Criggler-Najjar syndrome in one case, gall stones with intrahepatic dilatation in one case. This is in agreement with Chaabouni et al. (2007) who found that biliary cirrhosis due to biliary atresia was the most frequent etiology (40 %) followed by metabolic cirrhosis (17 %) and post- hepatitic cirrhosis (17 %). The same results were obtained by Delghani et al. (2007) and Larrosa-Haro et al. (2006) who found that biliary atresia was the most common cause of CLD in children (27.7, 27.8 % respectively).

Intestinal protozoa are increasingly being studied because of their association with acute and chronic diarrhea

Table 1 Distribution of etiology of CLD in GI

Etiology	No (50)	%
Biliary atresia	12	24
Chronic hepatitis B virus (HBV)	2	4
Autoimmune hepatitis	11	22
Chronic hepatitis C virus (HCV)	5	10
Metabolic causes	7	14
Budd-Chiari syndrome	4	8
Drug induced hepatitis	1	2
Hydatid disease	1	2
Choledochal cyst	1	2
Congenital hepatic fibrosis	2	4
Sclerosing cholangitis	2	4
Criggler-Najjar syndrome	1	2
Gall stones with intrahepatic dilatation	1	2

Table 2 Comparison between GI and GII as regards type of parasites detected by stool examination

Type of parasites	GI (50)		G II (50)		Statistical analysis	
	No	%	No	%	χ^2	*p* value
Unstained techniques						
E. histolytica/E. dispar	8	16	1	2	14.391	0.045*
G. lamblia	7	14	9	18		
B. hominis	7	14	5	10		
E. histolytica + *G. lamblia*	1	2	0	0.0		
E. histolytica + *B. hominis*	1	2	0	0.0		
G. lamblia + *B. hominis*	1	2	0	0.0		
B. hominis + *E. coli*	1	2	0	0.0		
Stained techniques						
Cryptosporidium	5	10	6	12	1.477	0.478**
Microsporidium	1	2	0	0.0		

* Significant; ** Not significant

Table 3 Comparison between GIa and GIb as regards results of stool examination

Stool examination	GIa (25)		GIb (25)		Statistical analysis	
	No	%	No	%	χ^2	*p* value
Unstained techniques						
+ve (26)	18	72	8	32	6.490	0.0108*
−ve (24)	7	28	17	68		
Stained techniques						
+ve						
Cryptosporidium (5)	3	12	2	8	1.200	0.549**
Microsporidium (1)	0	0.0	1	4		
−ve	22	88	22	88		

* Significant, ** Not significant

Table 4 Comparison between GI and GII as regards anthropometric measurements

	GI (50)		GII (50)		Total (100)		Statistical analysis	
	No	%	No	%	No	%	χ^2	*p* value
Weight percentile								
<25	19	38	10	20	29	29	8.416	0.038 *
25–50	11	22	7	14	18	18		
50–75	15	30	19	38	34	34		
>75	5	10	14	28	19	19		
Height percentile								
<25	23	46	10	20	33	33	14.148	0.003*
25–50	16	32	11	22	27	27		
50–75	5	10	13	26	18	18		
>75	6	12	16	32	22	22		

* Significant

in immunocompromised as well as immunocompetent patients. In these patients, parasites such as *Cryptosporidium parvum, Enterocytozoon bieneusi, Encephalytozoon intestinalis* and *Strongyloides stercoralis* may disseminate to other organs such as bile and liver ducts, producing symptomatology specific to the organ affected (Botero et al. 2003). In this study, intestinal parasitosis was diagnosed by examination of stool samples, using very simple methods in which the forms of the parasites were observed. However, there were some kinds of protozoan required special staining techniques for them to be recognized under the microscope. The diagnosis of cryptosporidiosis is based mainly on detection of the typical oocysts in stool specimens, by using of an acid-fast stain (Garcia et al. 1983) and diagnosis of intestinal microsporidiosis by use of modified trichrome stain (Ryan et al. 1993; Weber et al. 1992). To

Table 5 Correlation between weight percentile and parasitic infection in GI as regards results of stool examination

Stool examination	Weight percentile					Statistical analysis	
	<25	25–50	50–75	>75	Total	χ^2	*p* value
Unstained techniques							
No	11	4	6	5	26	8.760	0.033*
%	42.31	15.38	23.08	19.23	52.00		
Stained techniques							
No	1	3	1	1	6	3.614	0.306**
%	16.67	50.00	16.67	16.67	12.00		

* Significant, ** Not significant

Table 6 Correlation between height percentile and parasitic infection in GI as regards results of stool examination

Stool examination	Height percentile					Statistical analysis	
	<25	25–50	50–75	>75	Total	χ^2	*p* value
Unstained techniques							
No	10	9	4	3	26	2.491	0.477**
%	38.46	34.62	15.38	11.54	52.00		
Stained techniques							
No	1	4	0	1	6	5.064	0.167**
%	16.67	66.67	0.00	16.67	12.00		

** Not significant

Table 7 Correlation between liver function test (aspartate transferase, AST) and parasitic infection in GI as regards results of stool examination

Stool examination	AST			Statistical analysis	
	Normal	Abnormal	Total	χ^2	*p* value
Unstained techniques					
No	13	13	26	0.349	0.554**
%	50.00	50.00	52.00		
Stained techniques					
No	4	2	6	0.450	0.502**
%	66.67	33.33	12.00		

** Not significant

Table 8 Correlation between liver function test (alanine transferase, ALT) and parasitic infection in GI as regards results of stool examination

Stool examination	ALT			Statistical analysis	
	Normal	Abnormal	Total	χ^2	*p* value
Unstained techniques					
No	15	11	26	0.941	0.332**
%	57.69	42.31	52.00		
Stained techniques					
No	4	2	6	0.021	0.884**
%	66.67	33.33	12.00		

** Not significant

minimize laboratory costs, AFT stain was used in this study to demonstrate acid-fast oocysts of *Cryptosporidium* as well as microsporidial spores. This stain yielded results comparable to those obtained by modified ZN stain. An important clinical application is that stool examination might obviate the need for invasive techniques for diagnosis of intestinal protozoa. In addition, simplification of diagnostic procedure will facilitate determination of the prevalence and thus elucidate the pathogenic significance of intestinal parasites as a cause of chronic diarrhea in both immunocompromised and immunocompetent patients. This will facilitate monitoring of trials of treatment and improved the ability to follow the natural courses of the diseases (Hammouda et al. 1996).

In the present study, it was found that the most commonly intestinal protozoa identified in CLD patients in

order of frequency were: *E. histolytica/E. dispar* (16 %), *G. lamblia* (14 %), *B. hominis* (14 %), *Cryptosporidium parvum* (10 %), *E. histolytica* and *G. lamblia* (2 %), *E. histolytica* and *B. hominis* (2 %), *G. lamblia* and *B. hominis* (2 %), *B. hominis* and *Entamoeba coli* (2 %) *and Microsporidium* (2 %). There was no significant correlation between these parasitic infections and elevated liver function tests (AST and ALT). As compared to the controls, the observed incidence of these organisms in CLD patients was significantly higher ($p < 0.045$). Identification of these parasites in both CLD patients and controls is a reflection of poor environmental hygiene. Another interesting finding was the coinfection of *B. hominis* and *Entamoeba coli*. The recognition of non-pathogenic intestinal parasite, *Entamoeba coli* is a useful indicator of the level of fecal contamination (Hammouda et al. 1996). These multiple intestinal infections can aggravate the morbidity in CLD patients especially in young children.

Regarding parasitic infection, the obtained results were in concordance with those of Younes et al. (1996) who studied the incidence of recurrent diarrhea in patients with CLD and found *E. histolytica* in 21.5 % of cases and *G. lamblia* in 23 % of their cases. However, Hegab et al. (2003) who studied 80 CLD patients, their ages ranged from 6 months to 14 years and found that *G. lamblia* the most common organism which present in (45 %) of cases followed by *E. histolytica* (37.5 %), *B. hominis* (25 %) and *Microsporidium* (22.5 %).

Regarding *Cryptosporidium*, the obtained result (10 %) was in concordance with those of Shrestha et al. (1993) who assessed the role of parasitic infection in patients with chronic diarrhea in 30 cases with obstructive and non-obstructive hepatic lesions and found that 10 % of cases had *Cryptosporidium*. On the other hand, this result was disagreement with those of Younes et al. (1996) who found that no *Cryptosporidium* in the patients with CLD. Also, El-Okbi et al. (1992) studied *Cryptosporidium* in patients with schistosomal liver diseases and found no *Cryptosporidium* in their patients. The observed high incidence of *Cryptosporidium* (12 %) in the control group who are coming from similar environmental, social and economic background as that of CLD patients. This interesting finding helped us in tracking the source of infection pointing to water sources contaminated and practice of unhygienic habits.

In this study no cases were found infected with *Strongyloides stercoralis*, this was in concordance with Hegab et al. (2003) and disagreement with Gaburri et al. (1997) who studied the prevalence of intestinal parasites in patients with hepatic cirrhosis and found *Strongyloides* in (40.2 %) of alcoholic cirrhotic patients. This difference in the results could be attributed to different communities (their study was done in Brazil) and also to different etiology of CLD in their cases.

Childhood is the time of intense growth; it is the period in which the velocity of individual's growth had a rapid increase. This study showed that CLD affect the nutritional status of the patients as shown in significant decrease in weight percentile and height percentile in cases compared to controls. This is in concordance with Loguercio et al. (2000), who conducted a study to evaluate whether dietary intake influence plasma amino acid concentration in different etiological groups of cirrhosis and they found malnutrition was common among CLD patients that reflected in decreasing weight and height percentiles among them. In addition, the weight of studied patients was significantly affected by presence parasites as 57 % of patients with parasitic infection had weight below 50th percentile compared with those who had no parasitic infection. Also, the height of studied patients was affected by parasitic infection although not reaching a significant correlation. These results were expected as parasitic infections are thought to contribute to child malnutrition and micronutrient deficiency and protein loss through subtle reduction in digestion and absorption, chronic inflammation and loss of nutrients (Hesham et al. 2004). The results were in accordance with those of Shalabi (1991) and El-Baroudy et al. (1993). On the other hand, Kandeel (1998) did not find any effect of parasitic infections on children growth, but he attributed this to the recent, light intensity of infection or infection for a short period.

In conclusion, CLD affect the immunity of the patients as shown in significant increase in the incidence of intestinal parasites in cases compared to controls.

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