

## Comparison of Different Techniques for the Diagnosis of Parasitic Infections among Leukemic Children

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**ABSTRACT:** **Background/objectives:** Acute lymphocytic leukemia (ALL) is a worldwide problem, and it is more prevalent in children. As the chemotherapy is taken, the host defenses are altered and the patient becomes more liable to infection. This study aimed at determining the frequency of parasitic infections among children with ALL in relation to controls, and to evaluate the different techniques used in the diagnosis of these infections. **Methods:** The study was carried out in Alexandria University Children's Hospital at El-Shatby during one year. The study included 117 children with ALL, and same number of immunocompetent children as a control group. Stool, urine, cerebrospinal fluid (CSF), and blood samples were collected and prepared to be examined by different techniques. **Results:** The overall percentages of parasitic infections were 90.6% and 58.1% among leukemic children and controls, respectively. Microsporidiosis was the most prevalent infection, and *Cryptosporidium parvum* was the most common coccidial infection. *Microsporidium* was the only parasite detected in the CSF of leukemic children. The best technique was modified Ziehl Neelsen to detect coccidia, Trichrome stain for protozoa and Quick-Hot Gram-chromotrope stain for microsporidial infection. **Conclusions:** There was a high percentage of parasitic infections among leukemic children, and the results indicate that the combination of many techniques is more likely to be effective in the diagnosis of these infections.

**Key words:** Acute lymphocytic leukemia (ALL); Leukemic children; Parasitic infection; Diagnostic Techniques

### INTRODUCTION

Acute lymphocytic leukemia is a cause of malignancy in children, worldwide problem. It is the most common malignancy diagnosed in childhood, representing nearly one-third of all pediatric cancers.<sup>(1)</sup>

In Egypt, according to the National Cancer Registry, leukemia is the leading

constituting 36.7% of all cases of childhood cancer diagnosed annually.<sup>(2)</sup> Beside leukemia that causes anemia and neutropenia, the intake of

immunosuppressive chemotherapy impair the immune responses and render the

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subjects more liable to viral, bacterial, as well as opportunistic parasitic infections.<sup>(3)</sup>

In Alexandria and Turkey, studies on children with ALL reported parasitic infections in 40.6% and 47.3% respectively.<sup>(4,5)</sup> In Iran, it was reported that the percentages of parasitic infections were 40% and 2.8% among leukemic children and controls, respectively.<sup>(6)</sup>

The current study aimed at determining the frequency of parasitic infections among children with ALL in relation to controls, and to evaluate different techniques in diagnosing these infections.

## MATERIAL AND METHODS

The study was carried out in Alexandria University Children's Hospital at El- Shatby during one year. It is a comparative study, one hundred and seventeen children with ALL were enrolled in the study, they were from two to fourteen years. Same number of immunocompetent children were chosen as

matched by age and sex to represent the control group.

All the children were subjected to the following:

- 1- An interviewing questionnaire to collect data from the hospital's reports and mothers of the target children. It included socio-demographic data such as age, sex, residence, level of education, and occupation of their parents, housing conditions, and the presence of animals. Clinical data including general symptoms and local gastrointestinal tract symptoms were collected.
- 2- Stool samples were collected;
  - a- Fresh samples were prepared for agar plate culture technique<sup>(7)</sup> to detect the larvae of *Strongyloides stercoralis*, other portions were prepared to be stained by trichrome stain<sup>(8)</sup> to detect intestinal protozoa and Quick-Hot Gram-chromotrope stain (QHG)<sup>(9)</sup> to

detect *Microsporidia*.

- b- Preserved stool specimens in 10% formalin were prepared by formol ether concentration technique (FE)<sup>(10)</sup> to diagnose intestinal helminths, and by using modified Ziehl-Neelsen Technique (ZN)<sup>(11)</sup> to diagnose the different coccidia.
- 3- Cerebrospinal fluid (CSF) samples were collected from leukemic children only. Thin smears were stained by QHG<sup>(9)</sup> and Giemsa<sup>(12)</sup> stains to detect *Microsporidia* by both stains and *Toxoplasma gondii* by the latter stain.
- 4- Blood samples were collected and separated to sera to detect *Toxoplasma gondii* antibodies by using the enzyme linked immunosorbent assay (ELISA) (Toxo G480) test (Equipar kit for Toxoplasma IgG).<sup>(13)</sup>
- Data were tabulated and analysed using statistical package for social sciences (SPSS) version 11.0 computer software package.<sup>(14)</sup>

## RESULTS

Table 1 shows that the overall percentage of parasitic infections was higher among leukemic cases than that among controls (90.6% and 58.1% respectively). The difference was statistically significant, and the risk of infection was higher among cases relative to controls (OR = 6.94, CI = 3.22-15.29). Microsporidiosis was the most prevalent infection (60.7% of case vs 11.1% of controls).

This was followed by Giardia lamblia with percentages of 51.3% among leukemic children and 33.3% among the controls, *Entameba histolytica* / *dispar* (47% of cases vs. 30.8% of control) and *Entameba coli* (22.2% of cases vs. 0.9% of controls).

Concerning coccidial infections, the most commonly detected one was *Cryptosporidium* with percentages of 42.7% and 34.2% among leukemic children and controls, respectively, followed by

*Cyclospora* (22.2% vs. 24.8%), *Toxoplasma gondii* (13.3% vs. 6%) and finally *Isospora belli* (1.7% vs. 0.0%), respectively.

As regards helminthic infections, *Strongyloides stercoralis* showed the higher percentage among leukemic children (2.6%). *Enterobius vermicularis* and *Ascaris lumbricoides* were detected only in controls (5.1% and 6%, respectively).

*Microsporidium* was the only parasite detected in the CSF of leukemic cases with percentages of 54.7% and 53.7% by using Quick-Hot Gram-Chromotrope stain and Giemsa stain respectively. (Table 2)

Table 3 demonstrates that the percentage of parasitic infections by using trichrome staining technique for detecting the protozoa was higher than that by using formol ether and the difference was statistically highly significant. Also the percentage of coccidial infection by using MZN stain was higher than that by using formol ether and the difference was statistically significant. Quick-Hot Gram-Chromotrope showed that the percentage of infection by *Microsporidia* was significantly higher than by using MZN. Figures (1-6) Show some parasites by different stains

**Table (I): Percentages of different parasitic infections among leukemic cases and controls**

Parasitic infection	Leukemic cases n = 117		Controls n = 117		OR	95% Confidence Intervals		Test of sign.	
	No.	%♦	No.	%♦		Lower	Upper	x2	P
<b>Protozoa</b>									
- -Flagellates and amebae									
<i>G. lamblia</i>	60	51.3	39	33.3	1.11	0.45	2.72	0.06	0.801
<i>E. histolytica/dispar</i>	55	47	36	30.8	1.06	0.46	2.44	0.02	0.876
<i>E. coli</i>	26	22.2	1	0.9	25.50	3.46	523.44	18.71*	<(0.001)
<b>Subtotal</b>	78	66.7	52	44.4	2.50	1.42	4.40	11.70*	<(0.001)
- Microsporidia	71	60.7	13	11.1	12.35	6.22	24.51	62.47*	<(0.001)
- Coccidia									
<i>C. parvum</i>	50	42.7	40	34.2	0.87	0.30	2.45	0.09	(0.764)
<i>Cyclospora</i>	26	22.2	29	24.8	0.48	0.21	1.11	3.54	(0.060)
<i>I. belli</i>	2	1.7	0	0	-	-	-	1.58	(0.503)
<i>T. gondii</i>	15	13.3**	7	6	1.88	0.64	5.67	0.78	(0.38)
<b>Subtotal</b>	63	53.8	49	41.9	1.62	0.93	2.81	3.36	(0.07)
<b>Helminths</b>									
<i>S. stercoralis</i>	3	2.6	0	0	-	-	-	17.00	(0.001)
<i>E. vermicularis</i>	0	0	6	5.1	-	-	-	1.99	(0.515)
<i>A. lumbricoides</i>	0	0	7	6	-	-	-	2.55	(0.228)
<b>Subtotal</b>	3	2.6	14	12	0.19	0.04	0.75	7.68	(0.006)
Total +ve	106	90.6	68	58.1	6.94	3.22	15.29	160.44*	<0.001
Total -ve	11	9.4	49	41.9					
<b>Total</b>	117	100	117	100					

\* Percentages are calculated from total sample.

\*\* Percentages are calculated from 113 cases (4 cases were missing).

\* Significant at p < 0.05

**Table (2): Microsporidial infection detected in CSF of ALL children by two staining techniques**

<i>Microsporidia</i>	Leukemic children on therapy			
	Total No. examined		Total infected	
			No.	%
Quick Hot Gram Chromotrope stain	95		52	54.7
Giemsa stain	95		51	53.7

**Table (3): Percentages of intestinal parasites detected by different techniques in the whole samples (cases and controls)**

Parasitic infection	FE		MZN		Trichrome		QHG		Test of sign.
	No.	%♦	No.	%♦	No.	%♦	No.	%♦	
<i>Giardia</i>	50	21.4	33	14.1	76	32.5	3	1.3	147.78** (< 0.001)
<i>E. histo/dispar</i>	31	13.2	0	0	77	32.9	0	0	232.44** (< 0.001)
<i>E. coli</i>	5	2.1	0	0	25	10.7	0	0	80.39** (< 0.001)
<i>Crypto</i>	10	4.3	90	38.5	0	0	0	0	338.97** (< 0.001)
<i>Cyclo</i>	0	0	38	16.2	21	9	3	1.3	95.82** (< 0.001)
<i>I.belli</i>	0	0	2	0.9	0	0	0	0	8.01 (0.09)
<i>Microsporidia</i>	0	0	16	6.8	0	0	84	35.9	293.11** (< 0.001)
<i>E.vermicularis</i>	6	2.6	0	0	0	0	0	0	24.12** (< 0.001)
<i>Ascaris</i>	7	3	0	0	0	0	0	0	28.17** (< 0.001)
<i>Total infection</i>	89	38.03	105	44.9	120	51.3	84	36	

\* % calculated from total examined (n=234)

\*\* Significant at p < 0.01

FE= Formol ether MZN= Modified Zieh-Neelsen chromotrope

Q HG= Quick-Hot-Gram-

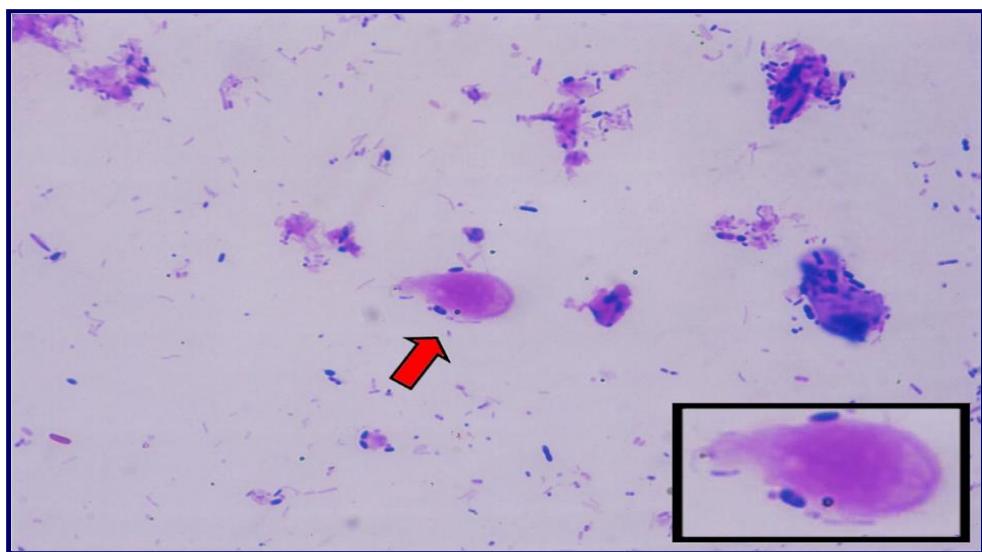


Fig. (1): *G. lamblia* (veg. form) by MZN (1000X)

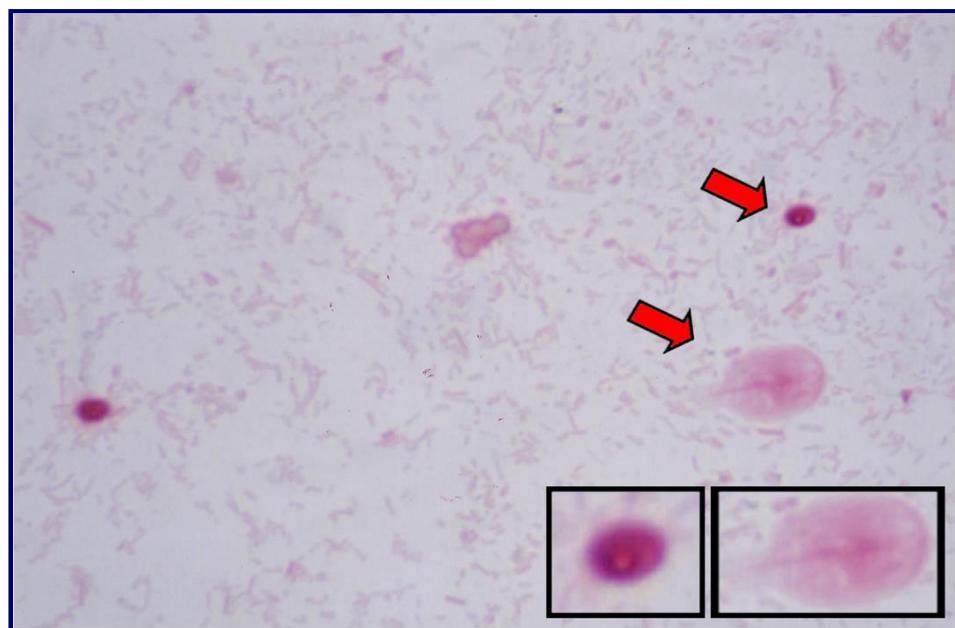


Fig. (2): *G. lamblia* (cyst and veg. forms) by trichrome stain (1000X)

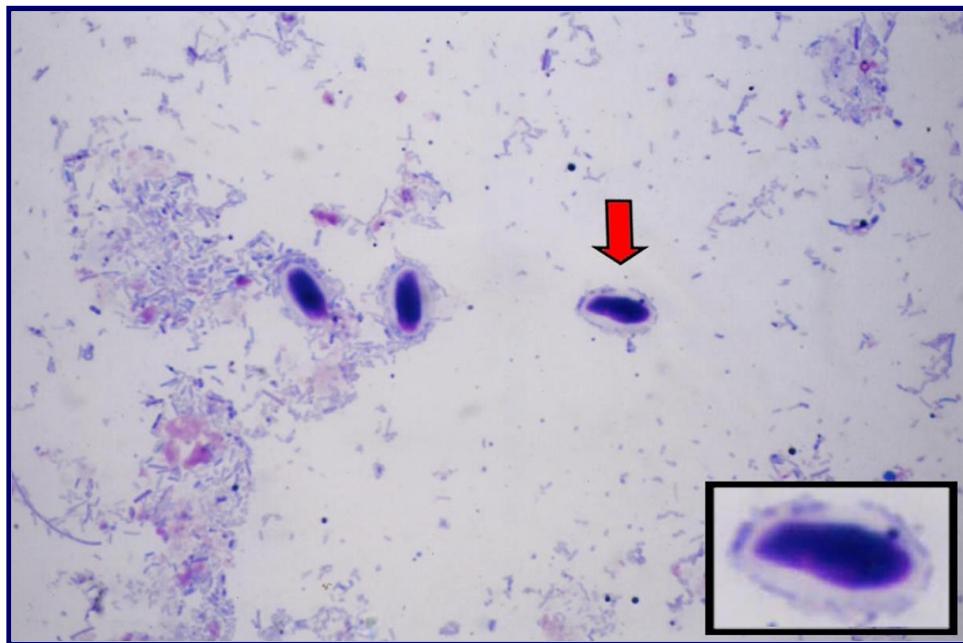


Fig. (3): *I. belli* by MZN (1000X)

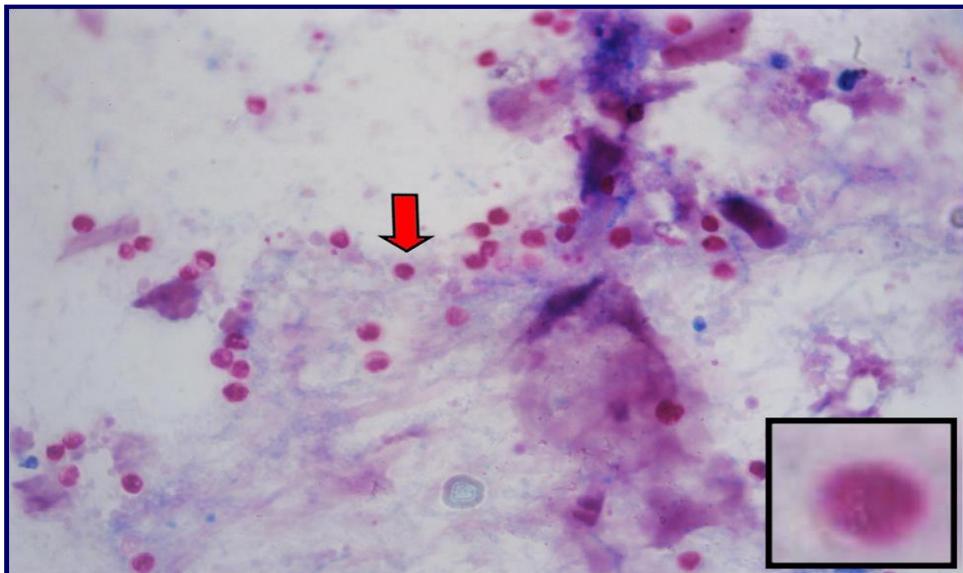


Fig. (4): *C. parvum* by MZN (1000X)

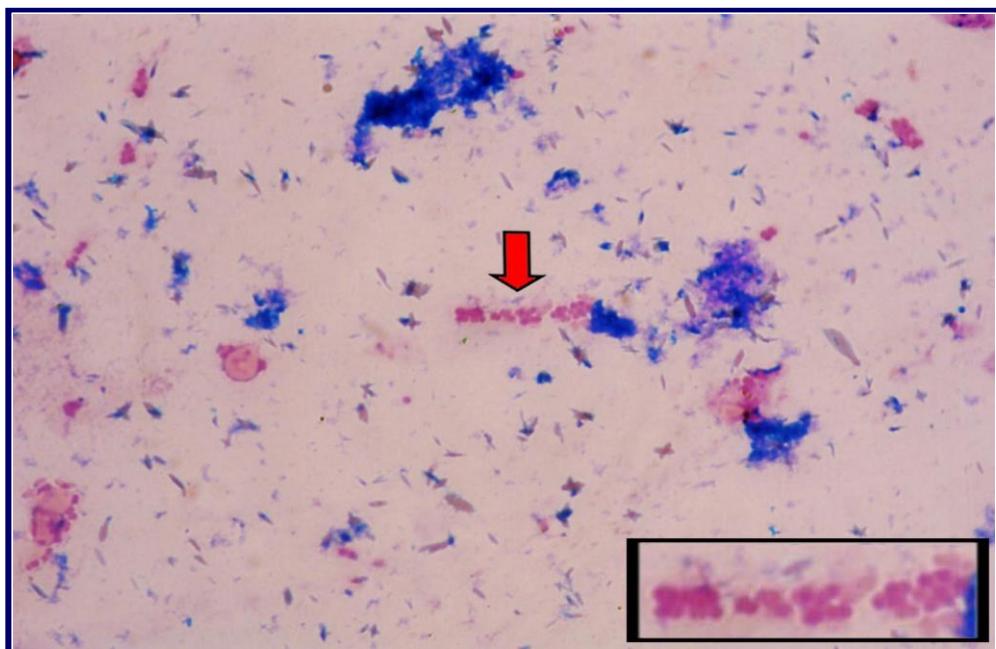


Fig. (5): *Microsporidia* by QHG in stool sample (1000X)

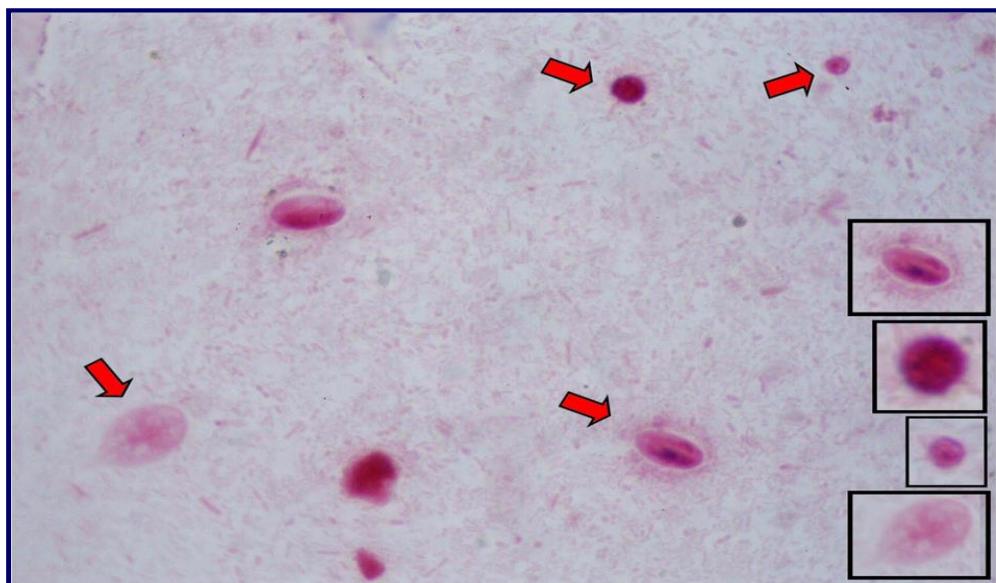


Fig. (6): *G. lamblia* (cyst & veg. forms, *Cyclospora* and *E. histolytica* cyst by trichrome stain (1000X)

## DISCUSSION

In the present study, several staining procedures were used to detect parasitic infections among leukemic children and controls. The high rates of infection observed in both groups may be due to the combination of different techniques done in the study which facilitate the diagnosis of different types of protozoa.

It was revealed that the overall percentage of infection was higher among cases than the controls; this might be attributed to the decrease of the immunological status of the leukemic children and their susceptibility to infection with opportunistic pathogens. Other studies reported high rates of parasitic infections among immunocompromised children in Egypt<sup>(15)</sup>, and other countries<sup>(5,6)</sup>.

In the present work, the protozoa were the most prevalent infection and helminths were the least. Conversely, a study in Nigeria reported that among AIDS patients, helminthic infections had higher rates than

protozoal infections<sup>(16)</sup>.

Different laboratory techniques in the present study proved that the MZN technique was a good reliable test for the identification of coccidia. Garcia and Bruckner<sup>(17)</sup> stated that the most effective technique in detecting *Cryptosporidium* is the MZN, and this was also proved by many authors<sup>(18,19)</sup>.

By comparing the different stains, the present study showed that the highest percentages of protozoal infections were reported by using trichrome staining technique (percentages of *G. lamblia*, *E. histolytica*, and *E. coli* were 32.5%, 32.9%, and 10.7% respectively), followed by formol ether concentration technique (same protozoa had percentages of 21.4%, 13.2%, and 2.1%, respectively). This can be explained because protozoa were less subjected to distortion by using trichrome technique, in addition, this stain had the ability for the identification of impeded

protozoa, even in the thicker smear. Idris and AL-Jabri<sup>(20)</sup>, revealed that the trichrome stain was superior to all other procedures in the detection of cysts and trophozoites of protozoa. Elkins<sup>(21)</sup> showed that trophozoites were difficult to be detected in feces particularly when they are no longer motile, and the cysts may be confused with pus cells or macrophages, hence it was found that trichrome stain helps to overcome these problems.

In the present study, the Quick-Hot Gram-Chromotrope staining technique was more sensitive in revealing *Microsporidia* with the highest percentage (60.7%) than that in the other techniques. A previous study demonstrated that the prevalence of *Microsporidia* in AIDS patients can exceed 20% by using the same technique<sup>(22)</sup>. Another study in the United States reported that the percentage of microsporidiosis was 15-34% among immunosuppressed adults<sup>(23)</sup>. *Microsporidia* have been underestimated in a previous study

(Goncalves da costa, 2000) due to the lack of application of accurate diagnostic methods<sup>(24)</sup>.

The results of this study indicate that the combination of many techniques is more effective in revealing parasitic infections among children with ALL.

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