CRYPTOSPORIDIOSIS AND IMMUNOLOGICAL STATUS IN CHILDREN WITH MALIGNANT DISEASES

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ABSTRACT

Objective: To investigate the relationship of Cryptosporidium and immunological parameters among children with malignant diseases.

Methods: Stool samples were collected from 101 children with malignant disease and 107 apparently healthy children. Direct smear method and then formalin-ether sedimentation method were done for all stool samples to identify intestinal parasites. Fecal smears were prepared from the sediment and stained by the modified Ziehl-Neelsen method for the recovery of acid-fast oocysts of Cryptosporidium. Phagocytic activity, complement C3 and C4 estimation, immunoglobulin levels and CD3, CD4, CD8, CD19 marking and phenotyping were carried out for 30 patients with acute lymphocytic leukemia (ALL) and 20 control group.

Results: ALL was the major type (47.52%) of malignant cases in the studied subjects. The other type ranged from 0.99% to 10.9%. Out of the 101 patients, 50(49.5%) were found to be positive for intestinal parasites compared to 13(12.15%) of the control group (P<0.01). Cryptosporidium oocysts were found to be excreted by 10(9.0%) patients and 1(0.93%) of the control group (P<0.01). The phagocytic activity, levels of IgM, IgA, IgG and CD3, CD4 cell numbers were lower in patients than in control group while higher in case of C3, C4, CD8 and CD19.

Conclusion: Children with malignancy are immunocompromised. Therefore, Cryptosporidium and other intestinal parasites must be considered in the differential diagnosis in this risky group in order to reduce the suffering often faced by those children.

INTRODUCTION

Cryptospridiosis is an emerging zoonotic disease which leads to intestinal and extra-intestinal diseases in both humans and animals. The major factor controlling the susceptibility to and severity of cryptosporidiosis appears to be the immune status of the host[1]. Those with great risk of infection including the immunocompromised patients[2,3]. They are including AIDS patients and those with various malignant, cytotoxic drugs receivers, prolonged corticosteriod therapy, the drugs used to prevent organ trasplant rejection[4], those with chronic diseases and persons who have congenital immunodeficiencies[4,5]. Since *Cryptosporidium* is an opportunistic parasite, this study is designed to determine the rate of cryptosporidiosis along with immunological status in children with malignant diseases.

MATERIALS AND METHODS

Subjects: One hundred and one patients with recently diagnosed malignancies according to a comprehensive diagnostic work up from typing of leukemia and staging for other malignancies who were admitted to Maternal and Child Hospital or attending out-patient *(Oncology center)* in Basrah Teaching Hospital for follow up. Their ages ranged from 9 month-16 years with mean age of 6.5±3.66 years. There were 62 males and 39 females. One hundred and seven apparently healthy children were involved in the study as a control group. Their ages ranged from 9 month-15 years with a mean of 7.1±3.32 years. There were 55 males and 52 females.

Stool examination: Direct smear method and then formalin-ether sedimentation concentration test[6] were carried out for the stool samples which were collected from all patients and control group to detect the diagnostic stages of the non-acid fast parasites. Fecal smears were prepared from the sediment and stained by the modified Ziehl-Neelsen method[6] for the detection of acid-fast parasite (*Cryptosporidium*).

Immunological status: It was carried out among 30 patients with acute lymphocytic leukemia (ALL) and 20 healthy control groups. Tests were performed within 2-3h after withdrawal of blood. Five ml of blood was drawn into ready-made 10 IU/ml heparin containing glass tube for the lymphocyte isolation in order to perform peripheral blood lymphocytes CD3, CD4, CD8 and CD19[7,8]. Lymphocytes were isolated by density gradient sedimentation and by procedure for lympocytes separation applied by Flow Laboratories Ltd, Scotland. Three ml of blood was obtained and serum was separated by centrifugation at 3000 r.p.m. for 5min and

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frozen at -20^{0}C until used for estimation of serum immunoglobulin and complement components by using radial immunodiffusion method[9,10].

Two ml of blood were collected and kept in vial containing EDTA anticoagulant for the measurement of phagocytic activity by using the Chemiluminescence assay[11].

Statistical analysis: Chi-sequared (X^2) test and where appropriate t-test were used. Differences were recorded as significant whenever the probability (P) was less than 0.05.

RESULTS

ALL was the major type (47.52%) of malignant cases in the studied subjects (Table-1). Other types ranged from 0.99% to 11.88%. Ages of most patients with ALL are within age group of 4-8 years (Table-1).

Table 1. *Number of cases in relation to type of malignant diseases with age and sex.*

Type of malignant cases	Age				Sex		Total	
	< 4	4-8	9-13	14-16	Male	Female	No.	%
Leukemia								
1. ALL	11	24	8	5	33	15	48	47.5
2. AML	-	4	3	-	2	5	7	6.93
3. CML	1	2	2	-	4	1	5	4.95
Lymphoma								
1. HL	-	7	3	1	5	6	11	10.90
2. NHL	4	4	3	1	7	5	12	11.88
Neuroblastoma	1	2	2	-	2	3	5	4.95
Retinoblastoma	1	-	-	-	-	1	1	0.99
Rhabdomyosarcoma	3	3	-	-	5	1	6	5.94
Ewing's sarcoma	-	1	1	-	2	-	2	1.98
Wilm's tumor	-	1	-	-	-	1	1	0.99
Adrenal carcinoma	-	1	-	-	1	-	1	0.99
Brain tumor	1	1	-	-	1	1	2	1.98
Total	22	50	22	7	62	39	101	100

ALL = Acute lyphocytic leukemia; AML = Acute myelocytic leukemia; CML = Chronic myelocytic leukemia; HL = Hodgkin lymphoma; NHL = Non-Hodgkin lymphoma.

The highest rate of *Cryptosporidium* infection among the 12 types of malignant diseases was found in patients with Hodgkin lymphoma (HL) (36.36%) (Table-2). However, other rates of infection were ranged from 6.25% among patients with ALL to 20% among patients with neuroblastoma. There were no any positive cases among other types of malignant diseases as shown in (Table-2).

Prevalence of various species of intestinal parasites (including *Cryptosporidium*) was 49.5% out of 101 stool samples. The highest rate of parasitic infections was observed in patients with HL (72.72%). However, there were no detectable cases of parasitic infection among patients with retinoblastoma, Wilm's tumor or adrenal carcinoma (Table-2).

Table 2. *Parasitic and Cryptosporidium infections among different types of malignant diseases in the studied subjects.*

Malignant disease	No. examined	Parasitic infections No. (%)	*Cryptosporidium* infection No. (%)
Leukemia			
1. ALL	48	22 (45.83)	3 (6.25)
2. AML	7	3 (42.86)	0
3. CML	5	3 (60.0)	0
Lymphoma			
1. HL	11	8 (72.72)	4 (36.36)
2. NHL	12	6 (50.0)	2 (16.70)
Neuroblastoma	5	3 (60.0)	2 (16.66)
Retinoblastoma	1	0	0
Rhabdomyosarcoma	6	3 (50.0)	0
Ewing's sarcoma	2	1 (50.0)	0
Wilm's tumor	1	0	0
Adrenal carcinoma	1	0	0
Brain tumor	2	1 (50.0)	0
Total	101	50 (49.5)*	10 (9.9)**
Control group	107	13 (12.15)*	1 (0.93)**

*X^2=63.000; P<0.01, **X^2=8.34; P<0.01

Males in both patient and control groups have higher parasitic infections than females (Table-3).

Table 3. *Distribution of parasitic infections among patients and control groups according to sex.*

Parasites	Patients		Control	
	Male	*Female*	*Male*	*Female*
Single infection:	24	16	7	6
Cryptosporidium	4	1	-	1
Giardia lamblia	4	4	3	1
Blastocystis hominis	7	6	2	2
Entamoeba histolytica	2	1	-	-
Enterobius vermicularis	3	2	1	-
Ascaris lumbricoides	1	-	-	-
Strongyloides stercoralis	-	1	-	2
Trichostrongylus colubriformis	1	-	1	-
Hymenolepis nana	1	1	-	-
Hymenolepis diminuta	1	-	-	-
Mixed infection:	5	5	0	0
Cryptosporidium, G.lamblia & B.hominis	-	1	-	-
Cryptosporidium & B.hominis	1	1	-	-
Cryptosporidium & G.lamblia	1	-	-	-
Cryptosporidium & E.vermicularis	1	-	-	-
G.lamblia & B.hominis	1	2	-	-
G.lamblia, B.hominis & H.diminuta	1	-	-	-
B.hominis & S.stercoralis	-	1	-	-
Total (%)	29 (28.7)	21 (20,8)	7 (6,5)	6 (5.6)

The mean value of phagocytic activity of patients was less than the control group and a significant difference was found (t=2.89, P<0.01) (Table-4). The relationship between immunoglobulin levels in both patients and control group showed a decrease in IgM, IgA, IgG levels in patients than in control but statistically not significant (P>0.05) (Table-4). There were elevated levels of both C3 and C4 in patients than control but they were statistically insignificant (P>0.05) (Table-4). Mean percentage values for CD markers of the ALL patients were significantly different from mean percentage value for CD markers of the control group, except the mean CD8 percentages, which expressed insignificant difference. Patients showed low CD3 and CD4 cell numbers while high in CD8, CD19 in patients than in control group (Table-4).

Table 4. *The immunological parameters among patient and control groups.*

Immunological parameter	Patients N = 30	Control N = 20	t-test
Phagocytic activity	0.936±1.56	2.004±0.6	2.89; P<0.01
Immunoglobulins:			
IgM	120.13±76.63	136.65±71.52	0.77; P>0.05
IgA	136.63±80.63	158.25±67.97	0.99; P>0.05
IgG	794.93±481.86	1018.1±397.98	1.72; P>0.05
Complements:			
C3	194.7±82.8	182.85±72.01	0.52; P>0.05
C4	48.95±14.5	42.47±18.34	1.39; P>0.05
CD markers:			
CD3	28.32±26.15	62.34±11.36	5.48; P<0,001
CD4	15.98±8.91	38.9±4.34	10.6; P<0.001
CD8	21.7±14.53	18.85±3.73	0.86; P>0.05
CD19	40.06±25.85	12.5±5.28	4.69; P<0.001

DISCUSSION

Leukemia is the most common type reported in this study. Similar result was observed in different studies[13,14]. Age group (4-8 years) was most involved, while other studies reported the peak age 4 years old[12,13]. The explanation for this result possibly due to the changing age structure of such cases with a shift towards the younger age group[15], or may be due to viral infection especially at this age group which lead to changes in immune system[13,16,17]. The etiology of tumor is not clear, but the relationship between exposure to ionizing radiation and cancer has been documented in various studies in other parts of the world as part from the survivors of atomic bombs in Hiroshima and Nagaszaki[18]. Also in 1991 and after use of depleted uranium (U238) by U.S. troops and their allies during their aggression in Iraq. This has been confirmed by detection of high radioactive using gamona spectrometric analysis of plants, water and soil samples taken from Basrah governorate, southern Iraq[19,20]. It would be predicted on the long run an increase in the incidence of cancer among children and in order to reduce the incidence, promoting in healthy life style such as increased intake of nutrients which contain anti-carcinogenic factors and reducing exposure to other risk factors, like smoking, chemical pollutants beside improvement in cancer screening program and early detection. Males were more affected than females by malignant diseases including leukemia. Similar result was obtained by two studies done in United States[12,13]. *Cryptosporidium* was found in 9.9% in different types of malignancy. Most of *Cryptosporidium* infection was reported in Hodgkin's lymphoma rather than other types of malignancy. These results indicate that patients with Hodgkin's disease have abnormalities of the cellular immune system (T-lymphocyte dysfunction) by the disease itself or by cytotoxic drug that heightens their risk for such infection[21]. This result was in agreement with a recent study done in Basrah city which found the incidence of cryptosporidiosis among cancer patients to be (8%)[22]. In another study done in Egypt, the incidence of cryptosporidiosis among cancer patients was (6.3%)[23], while in India only 0.3% of patients with malignancy have this infection[24]. In an earlier study also in India, the incidence of cryptosporidiosis among cancer patients was 1.3%[25]. Another study done in Australia did not find any case of cryptosporidiosis amongst cancer patients with diarrhea[26]. This variation in the incidence of cryptosporidiosis could be explained by the interaction with malnutrition[27], chemotherapy[21], other infections and animal contacts[24]. In this work, parasitic infections were found in 49.5% in different types of malignancy. Most of them were reported in Hodgkin's lymphoma (72.72%) than other types of malignancy. The resulted percentage (49.5%) of parasitic infections was higher than (23%) which was found by Abaza *et al*[23] in Egypt and

also higher than the reported by Rivera *et al,* who found only 12.9% of children with acute leukemia to have intestinal parasitosis[28]. The reduced phagocytic activity noticed among the leukemic patients may be due to neutropenia, which is most important deficiency phenomenon in the host defense occurring commonly in acute leukemia[13]. Cancer chemotherapy may also produce defects in neutrophil function, and combination of some drugs can produce a significant decrease in the phagocytic killing capability[21]. The low levels of IgM, IgA and IgG in sera of patients of this study might indicate the suppressed antibodies production by B-lymphocytes in response to particular antigens[29].

In this study, mean C3 and C4 values were elevated in patients with ALL than control group but statistically the difference was not significant. These complement components are not helpful indicative tools in patients with leukemia[30]. The use of immunologic markers to determine the degree of differentiation of leukemia has become an essential part of the diagnostic evaluation. This information is invaluable for selecting therapy and for determining prognosis and can help in the detection of relapse or of secondary leukemia[31]. In this study, CD3, CD4, CD8 and CD19 were chosen for their relationships with malignant diseases as well as microbial infections. CD4 was lower in patients than controls while CD8 was inverted which would make these patients are highly susceptible to get the infection by opportunistic pathogens including *Cryptosporidium.*

In Conclusion, Patients suffering from various malignancies present with varying degrees of immunodeficiency caused by their treatment or due to the disease process itself. Consequently, *Cryptosporidium* and other intestinal parasites must be considered in the differential diagnosis in patients with malignancy in order to reduce the suffering often faced by those patients.

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