Research Article

Parasites Detected in Immunocompromised Children in Pediatric Minia University Hospital

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Abstract
This was a cross sectional study, applied on 100 children (47 male and 53 female) their age ranged from 2-15 years, attended to the outpatients clinics of Paediatric Minia University Hospital, Minia District, 80 immuno-compromised patients and 20 immuno-competent (apparent healthy) children. They divided into five groups, 20 child each; uncontrolled diabetes mellitus (DM) type I **(Group I)**, chronic renal diseases **(Group II)**, haemolytic anaemia (HA) with repeated blood transfusion **(Group III)**, moderate to severe malnourished **(Group IV)** and apparently healthy children **(Group V)**. Stool and blood samples were collected from all the study groups. Stool samples were examined by different parasitological techniques; wet mount technique, concentration technique (Formal-Ether Concentration), staining with Giemsa stain, Modified Ziehl-Neelsen (ZN) stain and Stool culture for nematodes larvae (The Harada-Mori technique). Serum samples were tested for detection of anti-*Toxoplasma* gondii (IgM & IgG) antibodies by ELISA technique. The study revealed that the percentage of parasites detected in the immuno-compromised children were higher than the immuno-competent ones. e.g. *Blastocystis* spp. were found by direct wet mount technique in (69 cases 86%), *Cryptosporidium* spp. (61 cases 76%), *Microspor*a spp. (47 cases 59%), *E. histolytica/ E. dispar* (41 cases 51%), *Giardia lamblia* (32 cases 40%), *Cyclospora* spp. (30 cases 37.5%), *E. coli* (10 cases 12.5), *Storage mite* egg (5 cases 6.25%), *H.nana egg* (4 cases 5%), *Ascaris lumbricoides egg* (one case 1.25%) and *S. haematobium* egg (one case 1.25%). While among immuno-competent group, the parasites found were *Cryptosporidium* spp. (17 cases 72.5%), *Blastocystis* spp. (13 cases 65%), *Cyclospora* spp. (4 cases 20%), *Microspora* spp. (4 cases 20%), *E. histolytica/ E. dispar* (3 cases 15%) and *Giardia lamblia* (3 cases15%). Between different immuno-compromised children, it was found that parasitic infection in uncontrolled diabetes mellitus, chronic renal diseases and hemolytic anemia (HA) was more compared to moderate to severe malnourished children. *Toxoplasma gondii* antibodies were detected in 40% of children with uncontrolled diabetes mellitus, 90% of children with chronic renal diseases, 80% of children with (HA), 50% of malnourished children and 65% of immuno-competent ones by ELISA technique.

Keywords: *Blastocystis* spp.; *Cryptosporidium* spp.; Immuno-compromised, immuno-competent, Direct smear; Modified Ziehl-Neelsen (ZN); The Harada-Mori technique, ELISA.

Introduction
Parasitic diseases are one of the major causes of morbidity and mortality in more than 3 billion infected people worldwide, mainly in the developing countries (Kamki et al., 2015, El-Mahallawy et al., 2014). Children are the most common affected population (Chirdan et al., 2010).

Acquisition of infection, clinical severity and outcome of parasitic diseases often depend on innate and acquired host immunity (Evering and Weiss, 2006). An immune-compromised host is a patient who does not have the ability to respond normally to an infection due to impaired or weakened immune system. This inability to fight infection can be caused by a number of conditions including diseases (e.g. diabetes mellitus, renal diseases, malignant diseases, hematologic diseases, malnutrition and others), and drugs (e.g. long duration of corticosteroid therapy) (Hatzipantelis et al., 2013). This will establish a favourable condition for opportunistic parasites to flourish over the host system causing opportunistic parasitism (Samuel, 2016).

The commonest parasites causing morbidity and/or mortality in the immuno-compromised patients are parasites which inhabit the gastrointestinal tract (GIT) (e.g. *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Microspora* spp., *Isospora belli*, and *Strongyloides stercoralis*) and parasites of the reticulo-endothelial cells (e.g. *Toxoplasma gondii* and *Leishmania donovani*) (Baiomy et al., 2010, Noskin et al.,1997).

Thus, this study aimed to determine the incidence of various parasitic infections in stool samples of immune-compromised children attending the Pediatric Minia University Hospital by different techniques and comparing with immune-competent group. Also, to detect the anti-*Toxoplama gondii (*IgM & IgG) antibodies in serum samples of the study groups by ELISA technique.

Subjects and methods

Study design and setting

This was a cross sectional study, carried out on 100 children (47 male and 53 female), (80 immune-compromised children and 20 immune-competent apparent healthy children). Their age ranged from 2-15 years. They had been enrolled from Paediatric Minia University Hospital, Minia District, Egypt, during the period from April 2017 to January 2018.

They were classified into: Group I: 20 cases of uncontrolled diabetes mellitus type1, Group II: 20 cases of chronic renal diseases (nephrotic syndrome (NS) under chronic steroid therapy and chronic renal failure), Group III: 20 cases of haemolytic anaemia with repeated blood transfusion, Group IV: 20 cases of moderate to severe malnou-rishment and Group V: 20 cases of apparent healthy children.

Data collection

Patient's related demographic and clinical data were recorded using a prepared questionnaire including; Name, gender, age, residence, water supply and if received chronic steroid treatment or not.

Stool examination

Fresh stool samples were collected from each patient of the study groups in clean labelled plastic containers. The stool samples were transferred to the Laboratory of Parasitology Department, Faculty of Medicine, Minia University, to be examined immediately by different techniques for parasitic infections. Samples were examined macroscopically then direct wet mount (Saline and iodine prepa-rations) slides were prepared and microscopically examined. Concentration with Formal-Ether technique was used for the negative wet mount samples (WHO, 1991). All stool samples were permanently stained using Giemsa stain, (Garcia and Bruckner, 2001) and Modified Ziehl-Neelsen stain (Henriksen and Pohlenz, 1981). Also, stool culture for nema-tode larvae Harada- Mori technique was done for detection of *S. stercoralis*, hookworm, *Trichostrongylus* (Denham and Suswillo, 1995).

Serology

From each of the study groups, 2ml of venous blood were collected using sterile disposable syringes under aseptic precautions. Every specimen was labelled with patient name, date and time of collection. The blood samples were then transported to the Laboratory of Para-sitology Department, Faculty of Medicine, Minia University.

Serum was separated from whole blood by centrifugation at 3,000 rpm for 5 min, labelled, and kept in sterile micro tubes at -20˚C for further serological examinations. Each serum sample was tested for the presence of anti-*Toxoplasma* antibodies IgM and IgG using commercial ELISA kit (Cal Biotech Inc., Spring Valley, California, USA) following the manu-facturer's instructions. The results were read by optical density at 450 nm on an ELISA reader (Bio Tek Instruments, Inc Highland Park, Winooski, VT 05404-0998).

Ethical approval

The study protocol was approved by the scientific ethical committee of the Department of Parasitology, Faculty of Medicine, Minia University at their monthly meeting on March 2017. A written informed consent was obtained from the children's parents after full expla-nation of the purpose and technique of the study was given to them.

Statistical analysis

Data were coded revised and verified prior to data entry. Statistical analysis was done with software Statistical Package for the Social

Sciences (SPSS version 20), quantitative data presented as mean and standard deviation, qualitative data presented as chi square and ANOVA test. P-values less than 0.05($P < 0.05$) were considered statistically significant

Results

In this study, examination of stool samples by using **direct wet mount technique** revealed that 70 out of 100 samples (70%) were confirmed positive for different parasitic infections. However, this percent was increased to 83(83%) when the concentration technique (Formal-Ether Con-centration) used for negative samples, 88(88%) when Giemsa stain is used. The percent of positive samples was increased to 93(93%), when modified Ziehl-Neelsen (ZN) was used. Unfortunately all stool samples were negative for detection of nematodes larvae by culture on Harada-Mori technique.

I- Parasites detected among the studied groups by direct wet mount (saline and iodine). Out of 80 stool samples collected from immuno-compromised groups, *Blastocystis* spp. were detected in 59(74%), *Entamoeba histolytica/ E. dispar* in 34(42.5%), *Giardia lamblia* in 21(26%), *E. coli* 10(12.5%), *Storage mite* egg, *Ascaris lumbricoides* egg, *Hymenolepis nana* egg, and *Schistosoma haematobium* egg were found in 5(6.25), 1(1.25%), 4(5%), and 1(1.25%) respectively.

Among various immuno-compromised groups, *Blastocystis* spp. were detected in 18(90%), 16(80%), 17(85%), 8(40%) of uncontrolled diabetes mellitus (DM) type I **(GI)**, chronic renal diseases **(GII),** haemo-lytic anaemia (HA) **(G III)** and moderate to severe malnourished children **(G IV)** respectively.

Entamoeba histolytica/E. dispar was detected in 7(35%), 12(60%), 3(15%), 12(60%) of the same groups respectively. While *Giardia lamblia* was detected in 6 (30%), 6(30%), 3(15%), 6(30%) of the immuno-compromised groups respectively; *E. coli* was detected in 3(15%), 3(15%), 2(10%), 2(10%) respectively. *Storage mite* egg accidentally detected in 4(20%), 1(5%) of (**G I)** and (**G II)**. *Ascaris lumbricoides* egg only detected in 1(5%) of (G I), *H.nana* egg detected in 1(5%), 3(15%) of **(G I)** and **(G II)**. *S. haematobium* egg only detected in 1(5%) of **(G II)** as shown in Table (1). While among the immuno-competent group, *Blastocystis* spp. Infection was found in 10 (50%), *Entamoeba histolytica/ E. dispar* in 3 (15%), *Giardia lamblia* in one case (5%) and there is no infection with *E. coli*, *Storage mite* egg, *H.nana* egg, *Ascaris* egg and *S. haematobium* egg. It was found that the parasitic infection was higher in immune-compromised children than immunocompetent ones .This difference was statistically significant ($P < 0.001$) as shown Table (1)

Table (1): Parasites detected in stool samples of the studied groups by using direct wet mount technique.

Parasitic infections	Immuno-compromised					Immuno-competent
	G I (n = 20)	G II (n = 20)	G III (n = 20)	G IV (n = 20)	Total (n = 80)	(n = 20)
***Blastocystis* spp.**	18(90%)	16(80%)*	17(85%)*	8(40%)	59(74%)	10(50%)
***E. histolytica/E. dispar* cyst / Trophozoi**	7(35%)	12(60%)**	3(15%)	12(60%)**	34(42.5%)	3(15%)
***Giardia lamblia* cyst/ Trophozoite**	6(30%)*	6(30%)*	3(15%)	6 (30%)*	21(26%)	1(5%)
***E.coli* cyst/ Trophozoite**	3(15%)	3(15%)	2(10%)	2(10%)	10(12.5%)	0(0%)
***Storage mite* egg**	4(20%)*	1(5%)	0(0%)	0(0%)	5(6.25%)	0(0%)
***Ascaris lumbricoides* egg**	1(5%)	0(0%)	0(0%)	0(0%)	1(1.25%)	0(0%)
***H.nana* egg**	1(5%)	3(15%)	0(0%)	0(0%)	4(5%)	0(0%)
***S.hematobim* egg**	0(0%)	1 (5%)	0(0%)	0(0%)	1(1.25%)	0(0%)
Total	40	42	25[a,b]	28 [a,b]	135[#]	14

Data were statistically analyzed by one way ANOVA test followed by Chi-squared test. Data were reported as numbers (%)

[#] $P < 0.001$ vs immuno-competent group

[*] $P < 0.05$ vs immuno-competent group

[**] $P < 0.01$ vs immuno-competent group

[a] $p < 0.001$ vs uncontrolled DM group

[b] $p < 0.010$ vs chronic renal diseases

II- Parasites detected among the studied groups by using Giemsa stain.

By using **Giemsa stain**, other parasites were detected such as *Cyclospra* spp. and *Microspora* spp., which cannot be detected by direct wet mount technique and Concentration technique.

Out of 80 stool samples from immuno-compromised group, *Blastocystis* spp. was detected in 55(69%), *Cyclospora* in 27(34%), *Microspora* in 8(10%), *Entamoeba histolytica / E. dispar* in 14 (17.5%), *Giardia lamblia* in 25 (31%) and *E. coli* in 4 (5%).

Among various immuno-compromised groups, *Blastocystis* spp. were detected in 11(55%), 12(60%), 18(90%), 14(70%) of the four immune-compromised groups respectively while *Cyclospora* spp. were detected in 10(50%), 11(55%), 5(25%), 1(5%) of them respectively.

Microspora spp. were detected in 4(20%), 2(10%) and 2(10%) of **(G I)**, **(G III)** and **(G IV)**. *Entamoeba histolytica / E. dispar* were detected only in two immuno-compromised groups; **(G I)** and **(G II)** in 7(35%) and 7(35%) of cases. Giardia lamblia was detected in 8 (40%), 9(45%), 6(30%), 2(10%) of the four immuno-compromised groups respectively. *E. coli* was detected in 2(10%), 1(5%), 1(5%) of **(G II), (G III) and (G IV).**

While in the immune-competent group, *Blastocystis* spp. were detected in 11(55%), *Cyclospora* spp. in 4(20%), *Microspora* spp. in 1(5%), *Giardia lamblia* in 2(10%) . *E. coli* and *Entamoeba histolytica/ E. dispar* were not detected.

The study revealed that parasitic infection was higher in immuno-compromised patients than in immuno-competent ones. This difference was statistically significant (P <0.001). as shown in Table (2)

Table (2): Parasites detected in stool samples of the studied groups by using Giemsa stain:

Parasitic Infection	Immuno-compromised					Immuno-competent
	G I (n = 20)	G II (n = 20)	G III (n = 20)	GIV (n= 20)	Total (n = 80)	G V (n=20)
***Blastocystis* spp.**	11(55%)	12(60%)	18(90%)	14(70%)	55(69%)	11(55%)
***Cyclospora* spp.**	10(50%)*	11(55%) *	5(25%)	1(5%)	27(34%)	4(20%)
***Microsopra* spp.**	4(20%)	0(0%)	2(10%)	2(10%)	8(10%)	1(5%)
***E. histolytica / E. dispar* cyst / Trophozoite**	7(35%)**	7(35%) **	0(0%)	0(0%)	14(17.5%	0(0%)
***Giardia lamblia* cyst/ trophozoite**	8(40%) *	9(45%)*	6(30%)	2(10%)	25(31%)	2(10%)
E. coli cyst and Trophozoite	0(0%)	2(10%)	1(5%)	1(5%)	4(5%)	0(0%)
Total	40	41	32	20[abc]	133[#]	18

Data were statistically analyzed by one way ANOVA test followed by Chi-squared test. Data were reported as numbers (%)

[#]P < 0.001 vs immuno-competent group

[*]P < 0.05 vs immuno-competent group [**]P < 0.01 vs immuno-competent group

[a] P < 0.001 vs uncontrolled DM [b] P < 0.010 vs chronic renal diseases.

[c] P < 0.041 vs haemolytic anaemia with repeated blood transfusion

III- Parasites detected by using modified Ziehl-Neelsen (ZN) stain.

Using **modified Ziehl-Neelsen (ZN) stain** revealed that out of 80 samples collected from immuno-compromised group, *Cryptosporidium* spp. were detected in 61(76%), *Cyclospora* spp. *in* 8(10%), *Microspora* spp. 44(55%) while in the immuno-competent group, *Cryptosporidium* spp. 17(72.5%), *Cyclospora* spp. 3(15%), *Microspora* spp. 3(15%).

Among various immuno-compromised groups, *Cryptosporidium* spp. were detected in 12(60%), 17(85%), 15(75%), 17(85%) of the four groups respectively, *Cyclospora* spp. were detected in 3(15%), 4(20%), 1(5%) of **G I**, **G III** and **G IV** while *Microspora* spp. were

detected in 10(50%), 15(75%), 10(50%) and 9(45%) of the four groups respectively as shown in table (3).

The study revealed that there was difference between parasitic infection in immuno-compromised and immuno-competent groups examined by (ZN) stain. This difference was statistically significant (P<0.001) as shown in table (3).

Table (3): Parasites detected in stool samples of the studied groups by using modified Ziehl-Neelsen (ZN) stain:

Parasitic infection	Immuno-compromised					Immuno-competent
	G I (n = 20)	G II (n = 20)	G III (n = 20)	G IV (n = 20)	Total (n = 80)	G V (n = 20)
***Cryptosporidium* spp.**	12(60%)	17 (85%)	15(75%)	17(85%)	61 (76%)	17(72.5%)
***Cyclospora* spp.**	3(15%)	0(0%)	4(20%)	1(5%)	8(10%)	3(15%)
Microspora spp.	10(50%)*	15(75%)**	10(50%)*	9(45%)*	44(55%)	3(15%)
Total	25	32	29	27	113#	23

Data were statistically analyzed by one way ANOVA test followed by Chi-squared test. Data were reported as numbers (%)

#P < 0.001 vs immuno-competent group

*P < 0.05 vs immuno-competent gp. **P < 0.01 vs immuno-competent group

IV- Parasitic infection in the studied groups regarding age, gender, residence and source of drinking water.

In the present study, children were divided into two age groups, 63 preschool children, their age ranged from 2-6 years, and 37 school children their age ranged from 7-15, Among preschool children, all were positive for parasitic infection (50 were immuno-compromised and 13 were immuno-competent), and in school children, 30 of them were immuno-compromised (all cases were positive) and 7 were immuno-competent (6 positive and only 1 case negative).

So there was no significant statistical difference between the incidence of parasitic infection among children with different age (P value is insignificant). Table (4).

Also there was no statistically significant difference between the incidence of parasitic infection among male and female children (P value insignificant). Table (5).

Table (4): Parasitic infection among children of different age groups:

Age / group	2-6 years (n=63)		7-15 years (n=37)		P value
	Immuno-compromised	Immuno-competent	Immuno-compromised	Immuno-competent	
Positive	50	13	30	6	0.611
Negative	0	0	0	1	

Table (5): Parasitic infection among male and female:

Gender	Immunocompromised (80)		P value	Immunocompetent (20)		P value
	Male (37)	Female (43)	1.000	Male (10)	Female (10)	0.981
Positive	37	43		10	9	
Negative	0	0		0	1	

Regarding residence, *Blastocystis* spp. was the most common parasite among children from rural areas followed by *Cryptosporidium* spp., *Microsopra* spp., *E. histolytica / E. dispar*, *Giardia lamblia*, *Cyclospora* spp., *E. coli* and *H. nana* egg as shown in table (6).

Regarding drinking water source, *Blastocystis* spp. was the most common parasite transmitted by tap water followed by *Cryptosporidium* spp., *Microsopra* spp., *E. histolytica / E. dispar*, *Cyclospora* spp., *Giardia lamblia*, *E. coli* and *H. nana* egg as shown in table (6).

Table (6): Intestinal parasites among children regarding residence and water supply in the studied groups:

Parasites	Immuno-compromised(80)					Immuno-competent (20)				
	Residence		Water supply		Total	Residence		Water supply		Total
	Urban	Rural	Tap water	Under ground water		Urban	Rural	Tap water	Under ground water	
Blastocystis spp.	26	43	48	21	69(86%)	6	7	9	4	13(65%)
Cryptosporidium spp.	24	37	43	18	61(76%)	6	11	11	6	17(72.5%)
Cyclospora spp.	10	20	23	7	30(37.5%)	1	3	1	3	4(20%)
Microsopra spp.	10	37	36	11	47(59%)	1	3	3	1	4(20%)
Giardia cyst/ Trophozoite	12	20	19	13	32(40%)	0	3	1	2	3(15%)
E. histolytica / E. dispar cyst / Trophozoite	10	31	31	10	41(51%)	2	1	2	1	3(15%)
E. coli cyst and Trophozoite	3	7	8	2	10(12.5%)	0	0	0	0	.
Storage mite	3	2	5	0	5(6.25%)	0	0	0	0	.
Ascaris lumbricoides egg	1	0	1	0	1(1.25%)	0	0	0	0	.
H. nana egg	1	3	4	0	4(5%)	0	0	0	0	.

V- Sero-positivity of anti *Toxoplasma gondii* IgM / IgG antibodies by ELISA technique.

In the present study anti-*Toxoplasma gondii* IgM antibodies was detected in 15%, 45%, 10%, 20% and 25% of the study groups respectively.

While, anti-*Toxoplasma gondii* IgG antibodies were detected in 35%, 65%, 80%, 35% and 40% of all groups respectively.

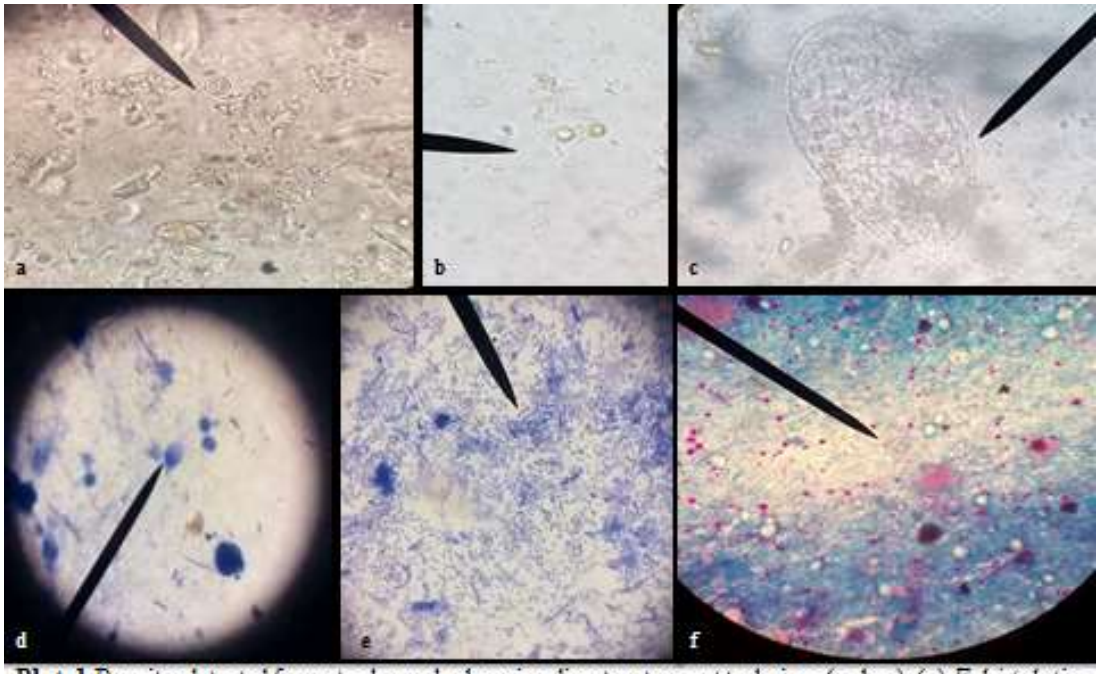
Moreover both anti *Toxoplasma gondii* IgM / IgG antibodies were detected in 4 children of chronic renal diseases group (G II), two children of uncontrolled diabetes mellitus (DM) type I (GI) and two children from haemolytic anaemia (HA) group (G III) and only one case in moderate to severe malnourished children (G IV) as shown in table (7).

It was found that the difference in detection of anti *Toxoplasma gondii* IgM and or IgG antibodies in children of G II, G III was statistically significant versus immune-competent group.

Table (7): Sero-positivity of anti *Toxoplasma gondii* IgM / IgG antibodies by ELISA technique:

No. of positive *T. gondii* Abs	Immune-compromised					Immuno-competent
	G I (n = 20)	G II (n = 20)	G III (n = 20)	G IV (n = 20)	Total (n = 80)	(n=20)
Positive IgM	3(15%)	9(45%)*	2(10%)	4(20%)	18(22.5%)	5(25%)
Positive IgG	7(35%)	13(65%)*	16(80%)*	7(35%)	43(54%)	8(40%)
Positive both IgG and IgM	2(10%)	4(20%)	2(10%)	1(5%)	9(11.25%)	0(0%)
Total positive anti *Toxoplasma* abs	8(40%)	18(90%)	16(80%)	10(50%)	52(65%)	13(65%)

*P < 0.05 vs immuno-competent group



Platel : Parasites detected from stool samples by using direct wet mount technique (a, b, c): (a) *E. histolytica* uni-nucleated cyst (X1000), (b) *Blastocystis* spp. (X1000), (c) *S. haematobium* egg (X400), Giemsa stain : (d) *Giardia lamblia* trophozoite, (e) *Microspora* spp., (X1000) and modified Z-N stain (f) *Cryptosporidium* spp. oocyst (X1000):

Discussion

Intestinal parasitic infections constitute a major public health problem, especially in developing countries, some of these parasites play an important role in triggering diseases in specific groups such as immuno-compromised individuals and young children (Ferreira, 2000).

The present study revealed that there was no difference between the incidences of parasitic infection among children with different age. This result could be explained by the majority of children (2/3) in this study were from age group (2-6 yrs.) while small number from age group (7-15 yrs.). this result is in a harmony with the results obtained by (Sehgal et al., 2010) and disagreed with (Wani et al., 2008, Khurana et al., 2005, Bansal et al., 2004, Fernandez et al., 2002, Albonico et al., 1999).

In the present study, there was no difference in the incidence of intestinal parasitic infection among males and females indicating that both gender are susceptible to infection as they exposed to the same environmental conditions like (playing, schools, drinking water sources …….. etc.). This result comes in agreement with the result of the study done by (Zabolinejad et al., 2013, Al—Megrin, 2010,

Zali et al., 2004) and disagreed with a study done by (Dhanabal et al., 2014, Baiomy et al.,2010, Abaza et al., 1995).

Regarding the residence of the patients, the current study found that children coming from rural areas had the largest number of intestinal parasitic infections compared to those coming from urban areas. This result comes in accordance with a study done by (Kiani H et al., 2016) This result was explained by (Tadesse, 2005, Okyay et al., 2004) who said that possible factors such as washing hands after defecation and certain demographic factors of mothers, such as literacy and occupation, may affect infection of the children with IPs. On the other hand, a study done by (Mulatu et al., 2015) found no difference in the prevalence of IPs among children from urban and rural areas.
Based on the source of the drinking water, the current study found that children using tap water had the largest number of intestinal parasitic infection compared to those consuming underground water. This result comes in a harmony with the results of a study done by (Idris et al., 2010). This may be due to either the contamination of drinking water during its passage in old water pipes or insufficient system of water treatment with chlorene.

By using different parasitological diagnostic techniques, the present study revealed that parasitic infection was high in immuno-compromised patients compared to immuno-competent ones. this result comes in accordance with studies done (Faidah et al., 2016, Hawash et al., 2015, Idris et al., 2010, Aksoy et al., 2003, Chokephaibulkit et al., 2001, Menon et al., 1999) and disagreed with a study done by (Fontanet et al., 2000).

Out of 80 stool samples collected from immuno-compromised groups, it was found that the highest parasite detected was *Blastocystis* spp. followed by *Cryptosporidium* spp., *Microspor*a spp., *Entamoeba histolytica/ E. dispar, Cyclospora* spp., *Giardia lamblia, E. coli, Storage mite, H.nana egg, Ascaris egg* and *S. haematobium* egg. These results are matched with other studies done by (Faidah et al., 2016, Mariam et al., 2008, Evering and Weiss, 2006, Zali et al., 2004, Yassien et al., 2001, Safar et al., 1996, Abaza et al., 1995). On the other hand, a study done by (Younes et al.,1996) didn't detect *Cryptosporidium* oocyst in stool samples of immunosupressed patients .
In the immuno-competent group, the parasites found were *Cryptosporidium* spp. followed by *Blastocystis hominis*, *Cyclospora* spp., *Microspora* spp., *Entamoeba histolytica/ E. dispar* and and *Giardia lamblia*, there is no infection with *E. coli*, *Storage mite*, *H.nana* egg, *Ascaris* egg *and S. haematobium* egg . A study done by (Mohandas et al., 2002) is matched with these results . Other study done by (Dhanabal et al., 2014) disagree with results of current study.

In the present study, among different immuno-compromised groups, in the first group, uncontrolled diabetes mellitus (DM) type I, it was found that the highest parasites detected was *Blastocystis* spp. followed by *Cryptosporidium* spp., *Cyclospora* spp., *Giardia lamblia* and *E. histolytica/ E. dispar*.. These results disagreed with (Bora et al., 2016), (Saxena et al., 1969), (Vinayak et al., 1967).

In the second group, children with chronic renal diseases, the parasites detected were *Cryptsporidium* spp., *Blastocystis* spp., *Microspora* spp., *E. histolytica/ E. dispar*, *Cyclospora* spp., and *Giardia lamblia*. These results come in accordance with a study done by (Abdel-Hafeez et al., 2012, Chonchol, 2006, Goetz et al., 2001, Descamps-Latscha and Chatenoud,1996, Massry, 1991).
In the third group, children with hemolytic anemia (HA) with repeated blood transfusion, it was found that the parasites detected were *Blastocystis* spp., *Cryptospordium* spp., *Microspora* spp., *Giardia lamblia*. This result disagrees by a study done by (Bora et al., 2016, Dori et al., 2011, Rao et al., 2003, Tsuyuoka et al., 1999).

In the fourth immuno-compromised group, moderate to severe malnourished children, the parasites detected were *Cryptsporidium* spp., *Blastocystis* spp., *E. histolytica/ E. dispar*, *Microspora* spp. and *Giardia lamblia*. This results comes in a harmony with result of a study done by (Abdel-Hafeez et al., 2012) and

disagreed by (Bechir et al., 2012, Bhandari et al., 1985).

It was found that parasitic infection in uncontrolled diabetes mellitus (DM) type I, chronic renal diseases and hemolytic anemia (HA) with repeated blood transfusion more than moderate to severe malnourished children. This difference might be related to difference in the immunological status of studied groups, their susceptibility to infection and different environmental factors (Goodgame,1996).
The present work revealed that the use of different diagnostic techniques led to a higher sensitivity for detection of parasites. Regarding concentration technique, the use of formalin fixes and preserves the fecal specimen and ether decreases the specific gravity of small fecal particles causing them to float in the suspension. The coarse nonabsorbent elements including eggs and cysts are left at the bottom, and ether also dissolves fat, the addition of these two chemicals and centrifugation improved the isolation rate.

In the present study, the use of modified Zeihl-Neelsen (ZN) stain was the beneficial for identification of coccidian protozoan parasites in stool samples with positive results 93%. This result comes in agreement with the results reported by (Ali et al., 2000, El-Naggar et al.,1999) but in the present study, *Isospora belli* cannot be detected. This result agrees with results of (El-Naggar et al., 1999, Safar et al., 1996, Abaza et al., 1995) who attributed the rare occurrence of Isospora belli in their study to host immune response which is unknown (Markell et al., 1984) or might be due to environmental factors.

In the current study, all stool samples were negative for detection of nematodae larvae by culture on Harada- Mori technique. This could be explained by that there were no detected eggs/ larvae (hook worms, *Strongyloides stercoralis*) in stool samples by direct wet mount technique (Saline and iodine preparation) and Concentration techniques (Formal-Ether Concentration). This result is in accordance with a study done by (Baiomy et al., 2010). This could be explained by the ability of larvae to penetrate the intestinal mucosa and to cause hyperinfection especially in the immune-suppressed hosts limited the number of excreted larvae (Oliver et al., 1989). For diagnosing strongyloidiasis, multiple stool samples examination was suggested (Nielsen and Mojon, 1987) to avoid false negative results.
The present study showed no difference in incidence of *T. gondii* infection among the immuno-compromised group (65%) and the immuno-competent group (65%). These results are in a harmony with other previous studies done by (de la Luz Galvan-Ramirez et al., 2012, Sukthana et al., 2000). On the other hand, this result disagrees with a study done by (Baiomy et al., 2010) who detected Anti-*Toxoplasma* antibodies in (6%) of immuno-compromised patients and none in controls.

Among the immuno-compromised group, anti-*Toxoplasma gondii* antibodies were detected in 65%; 22.5% were seropositive for IgM antibodies which indicates acute infection, 54% were seropositive for only IgG antibodies which indicate chronic infection, and 11.25% were seropositive for both IgM and IgG antibodies which indicates possible recent infection in the last 12 months. These results are in concordance with other similar studies done by (Molan and Rasheed, 2016, Moghimi et al.,2015, Rahimi et al., 2015, Al-Najjar and Al-Mukhtar,2009, Afify and Morsy, 1996).
Among the immuno-competent group, anti-*Toxoplasma* gondii antibodies were detected in 65%; 40 % were seropositive for IgG antibodies and 25% were seropositive for IgM antibodies. These results are supported by other studies done by (El-Geddawi et al., 2016, Fan et al., 2012, Elsheikha et al., 2009, Reda et al., 1996, Griffin and Williams, 1983).

Conclusion

The study revealed the following:

1- The number of parasites detected in the immuno-compromised children was higher than the immuno-competent ones. Parasites found among immuno-compromised children were *Blastocystis* spp. by direct wet mount technique (69 cases 86%), *Cryptosporidium* spp. (61 cases 76%), *Microspora* spp. (47 cases 59%), *E. histolytica/E. dispar* (41 cases 51%), *Giardia lamblia* (32 cases 40%), *Cyclospora* spp. (30 cases 37.5%), *E. coli* (10 cases 12.5), *Storage mite* egg (5 cases 6.25%), H.nana egg (4 cases 5%), *Ascaris lumbricoides* egg (one case

1.25%) and *S. haematobium* egg (one case 1.25%).

2- Among immuno-competent group, the parasites found were *Cryptosporidium* spp. (17 cases 72.5%), *Blastocystis* spp. (13 cases 65%), *Cyclospora* spp. (4 cases 20%), *Microspora* spp. (4 cases 20%), *E. histolytica/ E. dispar* (3 cases 15%) and *Giardia lamblia* (3 cases15%).

3- Between different immuno-compromised children, it was found that parasitic infection in uncontrolled diabetes mellitus (DM) type I, chronic renal diseases and haemolytic anaemia (HA) more compared to children with moderate to severe malnourished children.

4- As regard to ELISA technique, positive *Toxoplasma gondii* antibodies were detected in 40% of children with uncontrolled diabetes mellitus (DM) type I, 90% of children with chronic renal diseases, 80% of children with haemolytic anaemia (HA) with repeated blood transfusion, 50% of malnourished children and 65% of immuno-competent ones.

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