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IMPACT OF INTESTINAL PARASITES ON HEMATOLOGICAL PARAMETERS AMONG SCHOOL CHILDREN IN GHARBIA GOVERNORATE, EGYPT

By

AHMED M. S. BAYOUMY[1*], KHAIRY A. M. HASSAN[1], MORSY R. GENEIDY[1], and ALAA G. S. METAWEA[2]

Departments of Medical Parasitology, Faculties of Medicine (boys)[1] and (Damietta)[2]
Al-Azhar University, Egypt (*Correspondence: drahmedbayoumy@azhar.edu.eg)

Abstract

Generally, the intestinal parasites are responsible for morbidity in school-aged children worldwide, especially in undeveloped countries. So a cross sectional random study was conducted from early October, 2017 to late December, 2017 and included 200 children attending primary schools in Gharbia Governorate, Egypt. All cases were subjected to (1) a questionnaire filled out by an interview with the child and/or one of his/her parents. (2) Fresh stool samples were taken for parasitological diagnosis and detection of intestinal parasites by direct smear method, simple floatation and formol-ether concentration techniques. (3) Blood samples were collected for estimation of blood indices (CBC) and serum ferritin level. Among the total 200 (100 males and 100 females) cases included in the present study, 96 (48%) (47 males and 49 females) were proved to be positive for parasitic infection. The prevalence rate of the intestinal parasites among studied children was; *E. histolytica/dispar,* 56 cases (58.33%) which was the most common parasite found. The other infections were: *G. lamblia,* 8 (8.33%) cases, *S. mansoni*, 6 (6.25%) cases, *H. nana,* 6 (6.25%) cases, *E. vermicularis,* 4 (4.16%) cases and poly parasites in16 (16.66%) cases. Distribution of hematological indices among children was: Total 110 cases had anemia; 51 males (34 infected and 17 non-infected) and 59 females (38 infected and 21 non-infected). No children had severe anemia and iron store of them was adequate. Among 96 cases infected; 50 cases (52.08%) had normocytic normochromic anemia, 22 cases (22.91%) had microcytic hypochromic anemia and 24 cases (25%) were not anemic. There was a strong association between *G. lamblia* infection and presence of anemia as it was noted that all the 8 infected cases had anemia while 6 cases (75%) of them showed decrease in serum ferritin level. Among the 56 cases that had *E. histolytica/dispar* infection, 42 of them were anemic, while among the 6 cases of schistosomiasis mansoni, 4 cases of them were anemic. Regarding the 6 cases infected with *H. nana,* 4 cases of them were anemic while among 16 cases of mixed parasitic infection, only 14 cases were anemic. It was also noted that all the 4 cases suffering from *E. vermicularis* infection were not anemic.

Only 13 cases suffering from parasitic infections had low serum ferritin. Four of them had moderate type while the other 9 children had mild type. Non infected children had normal ferritin level. It is of interest to found that; 3 cases infected with *G. lamblia* had moderate decrease in serum ferritin level < 8ng/ml. One case had mixed infection had moderate decrease in serum ferritin level.

In conclusion, the present study showed a high prevalence of intestinal parasitic infection correlated with anemia which calls for a comprehensive public health intervention and regular parasitic screening in school-aged children.

Key words: intestinal parasitic infections, school children, blood indices, serum ferritin.

Introduction

Infection with intestinal parasites is endemic worldwide as a major public health problem among school-aged children in developing countries especially hot and humid environments. (Dash *et al,* 2010, Oliveira *et al*, 2015) where prevalence rates range from 30-60%, as compared to about 2% in the developed countries (Shubair *et al*, 2000; Brito *et al,* 2003).

In Egypt, the prevalence rate was depending on the socio-economic level, poor sanitary and environmental conditions, inadequate personal hygiene, absence of safe drinking water supplies and climatic factors as well as poor housing conditions (Abou El-Soud, 2009; Bayoumy *et al*, 2016; 2018). Intestinal parasitosis among school children has detrimental effects on the survival, appetite, growth and physical fitness (Ekpen-

yong, 2008; Jardim-Botelho *et al*, 2008), school attendance (Nematian *et al*, 2008) and cognitive performance (WHO, 2006).

Anemia affected half the school-age children and adolescents in developing countries (Huong *et al*, 2007). It has complex etiological factors, including micronutrient deficiencies (iron, folate, riboflavin, vitamin A & B12), hemoglobinopathies and parasitic infections (Midzi *et al*, 2010). Parasitic infections may influence nutritional and iron status by reducing nutrient intake and interfering directly or indirectly with iron absorption and transportation (Yanola *et al*, 2018). The presence of intestinal parasites was usually parallel with appearance of iron deficiency anemia (Ferreira *et al*, 2002). In school-aged children, both infections and iron deficiency can lead to anorexia. Infections inhibited the absorption of iron from the gastro-intestinal tract and iron deficiency lowers immunity to infections, which created a vicious circle of inadequate nutrition (Stoltzfus *et al*, 1997; Olsen *et al*, 1998).

The present study aimed to determine the prevalence of intestinal parasitic infections among school children in Gharbia Governorate, Egypt and explored the possible relationship between these infections and different hematological parameters and serum ferritin.

Materials, Subjects and Methods

A cross-sectional random study was conducted on 200 school children aging (6-12 years) attending primary schools in El-Santa City (50 students) and El-Santa villages (150 students) in Gharbia Governorate, Egypt during the period from early October, 2017 to late December, 2017 after permission from the local Education Authority. Exclusive criteria included pupils below 6 years as well as above 12 years, blood diseased and sick, febrile pupils at the time of the study. Also children who had taken anti-helminthic drugs within 6 months of the study or those who received blood transfusion or iron containing medicine within 3 months proceeding to the study were excluded.

All children were subjected to the following: (1) a questionnaire filled out by an interview with the child and/or one of his/her parents. (2) Fresh stool samples were collected in clean, labeled and wide mouthed plastic containers that had tight fitting overlapping lids. (3) Collection of aseptically 3 ml of venous blood samples for complete blood count (CBC) examination using Mindray BC-5800 auto hematology analyzer and another 2 ml of blood were collected for sera extraction. Serum ferritin concentration was analyzed by enzyme-linked immune fluorescent assay using the VIDAS® human ferritin enzyme immunoassay test kit (France).

Stool samples were examined macroscopically and microscopically by: (i) direct wet smear method. (ii) Concentration methods by simple floatation and formol ether concentration techniques **(WHO, 1994)**. The obtained data were tabulated and statistically analyzed.

The study was approved by the Governmental Health and Education Authorities and only children whose parents/guardians signed the informed consent were included in the present work.

Results

According to age and sex the randomly chosen 200 children were distributed as follows; 50 children aged from 6 to <8 years (23 males and 27 females), 80 children aged from 8 to <11 years (39 males and 41 females) and 70 children aged from 11 to 12 years (38 males & 32 females). $\chi^2 = 0.884$, $p = 0.643$ (Tab. 1).

Out of 200 stool samples examined, 96 (48%) were positive for intestinal parasites and the distribution of parasitic infections according to age group was presented as 23/50 pupil aged 6- 8 y were infected and 42/80 pupil aged 8- 11 y were infected while 31/70 pupil aged 11-12 y were infected; $\chi^2 = 1.116$, $p = 0.572$ (Tab. 2). Among the students; 51 males had low hemoglobin level compared to 59 females and 47 males have low PCV compared to 48 females while 19 males had low MCH compared to 7 females

with a significant differences as to MCH (p=0.01) (Tab. 3).

Out of 51 anemic males 34 of them were positive for parasitic infection while out of 59 anemic females 38 of them were infected. P=0.004, χ^2=11.011* (Tab 4). Among the 110 anemic children, 102 of them had hemoglobin level ranging between 10-11.5 g/dl and 66 were positive for intestinal parasitic infection compared to 8 pupils who had 8- <10 g/dl of hemoglobin and 6 of them were infected. According to type of anemia, 26 children had microcytic hypochromic anemia and 22 of them were positive for intestinal parasitic infection while 84 children had normocytic normochromic anemia and 50 were infected (Tab. 5).

Out of 96 children infected with different intestinal parasites; 72 (75%) were anemic while out of 104 non- infected pupils; 38 (36.5%) had anemia with significant statistical analysis; χ^2= 29.837, p= 0.01 (Tab. 6). Among the 96 cases having intestinal parasitic infection; 24 (25%) were not anemic, while 50 (52.08%) had normocytic normochromic anemia and 22 (22.9%) cases had microcytic hypochromic anemia (Tab. 7).

Among the 56 *E. histolytica/dispar* cases 42 (75%) were anemic and ten had microcytic hypochromic anemia. Eight giardiasis cases were anemic; six were related to microcytic hypochromic type. Regarding schistosomiasis and hymenolepiasis (6 cases of both), four had normocytic normochromic anemia. Four *E. vermicularis* cases were not anemic while of mixed infection 14/16 (87.5%) were anemic and 6 suffered from microcytic hypochromic anemia; χ^2= 16.444*, p=0.006 (Tab. 8).

Among the 56 *E. histolytica*; cases 24, 42, 40, 10 & 10 had low RBCs, Hb%, PCV, MCV & MCH ratio respectively. Among 8 *G. lamblia* cases 4, 8, 6, 6 & 6 had low RBCs, Hb%, PCV, MCV & MCH ratio respectively. Among 6 *S. mansoni* cases 4, 4 & 4 had low RBCs, Hb% and PCV ratio respectively while among 6 *H. nana* cases 2, 4 & 4 had low RBCs, Hb% & PCV ratio respectively. Among 16 mixed parasitic infection cases 4, 14, 14, 6 & 6 had low RBCs, Hb%, PCV, MCV & MCH ratio respectively. Four *E. vermicularis* cases had normal hematological parameters (Tab. 9).

No significant changes in total leucocytic count, number of lymphocytes, monocytes, neutrophils and basophils between infected and control (Tab.10).

Only the 13 cases suffering from parasitic infections had low serum ferritin. Four of them had moderate type while the other 9 children had mild type. Non infected children had normal ferritin level; χ^2= 15.062*, p= 0.01 (Tab. 11).

Three *G. lamblia* cases had moderate decrease in serum ferritin level <8ng/ml. One case had mixed infection had moderate decrease in serum ferritin level; χ^2= 40.640*, p= 0.001 (Tab. 12)

Table 1: Age and sex distribution among studied children

Age	6 - <8 y 50 (25%)		8 - <11 y 80 (40%)		11 – 12 y 70 (35%)		Total
	Male	Female	Male	Female	Male	Female	200
No. of cases	23	27	39	41	38	32	
%	46%	54%	48.8%	51.2%	54.3%	45.7%	

Table 2: Distribution of parasitic infection among infected children according to age group

Parasite species	6 - <8 y No. 50		8 - <11 y No. 80		11-12 y No. 70		Total	
	No.	%	No.	%	No.	%	No.	%
E. histolytica/dispar	11	11.5	26	27.1	19	19.8	56	58.3
G. lamblia	2	2.1	4	4.2	2	2.1	8	8.3
S. mansoni	0	0.0	1	1.0	5	5.2	6	6.3
H. nana	2	2.1	3	3.1	1	1.0	6	6.3
E. vermicularis	3	3.1	1	1.0	0	0.0	4	4.2
Mixed infection	5	5.2	7	7.3	4	4.2	16	16.7
Overall prevalence	23	24	42	43.7	31	32.3	96	100

Table 3: Distribution of the hematological indices among the examined children versus sex

		Hemoglobin level		Total	PCV		Total	MCH		Total
		Low	Normal		Low	Normal		Low	Normal	
Male	No.	51	49	100	47	53	100	19	81	100
	%	51	49	100	47	53	100	19	81	100
Female	No.	59	41	100	48	52	100	7	93	100
	%	59	41	100	48	52	100	7	93	100
χ^2	1.293			0.020			6.366*			
P	0.856			0.887			0.01			

Table 4: Distribution of infection among anemic males and females

Infection +/- / Anaemic cases	Infected		Non infected	
	No.	%	No.	%
Males (No. = 51)	34	66.7	17	33.3
Females (No. = 59)	38	64.4	21	35.6
Total (No. = 110)	72	65.5	38	34.5

Table 5: Correlation between anemic cases and intestinal parasitic infection

		Anemic cases (N= 110)	Positive parasitic cases (N= 72)
Hemoglobin level	8- <10 gm/dl	8	6
	10-11.5 gm/dl	102	66
Type of anemia	Microcytic hypochromic	26	22
	Normocytic Normochromic	84	50

Table 6: Distribution of anemia among infected and non-infected children

	Anemic		Normal		Total	
	No.	%	No.	%	No.	%
Infected cases (N= 96)	72	75.0	24	25.0	96	100%
Non infected cases (N = 104)	38	36.5	66	63.5	110	100%
Total (N= 200)	110	55.0	90	45.0	200	100%

Table 7: Distribution of type of anemia among infected children

Anemia (Absent / Present)		Positive cases (96)
Non anemic		24 (25%)
Anemic	Normocytic normochromic anemia	50 (52.08%)
	Microcytic hypochromic anemia	22 (22.9%)

Table 8: Distribution of anemia among infected children

Parasite	Positive cases	Anemic cases		
		Normocytic normochromic	Microcytic hypochromic	Total
E. histolytica/dispar	56	32	10	42 (75%)
G. lamblia	8	2	6	8 (100%)
S. mansoni	6	4	0	4 (66.7%)
H. nana	6	4	0	4 (66.7%)
E. vermicularis	4	0	0	0 (0%)
Mixed infection	16	8	6	14 (87.5%)
Total no.	96	50	22	72 (75%)

Table 9: Average levels of erythrocytes, hemoglobin levels, PCV, MCV and MCH among infected individuals

Parasite	No. of cases	RBCs		Hb%		PCV		MCV		MCH	
		N	L	N	L	N	L	N	L	N	L
E. histolytica/disper	56	32	24	14	42	16	40	46	10	46	10
G. lamblia	8	4	4	0	8	2	6	2	6	2	6
S. mansoni	6	2	4	2	4	2	4	6	0	6	0
H. nana	6	4	2	2	4	2	4	6	0	6	0
E. vermicularis	4	4	0	4	0	4	0	4	0	4	0
Mixed infection	16	12	4	2	14	2	14	10	6	10	6
Chi-square test	χ^2	6.596*		16.444*		12.043*		21.081*		19.780*	
	P	0.252		0.006		0.034		0.001		0.001	

N= Normal, L= Low, No. = number

Table 10: Average levels of WBCs, eosinophils, basophils, monocytes, lymphocytes and neutrophils in infected & non-infected children

	WBCs		Eosinophils		Basophils		Monocytes		Lymphocytes		Neutrophils	
	No.	high	No.	high	No.	high	No.	high	No.	high	No.	high
Non infected (104)	102	2	104	0	104	0	104	0	104	0	104	0
Infected (96)	92	4	65	31	94	2	96	0	78	18	84	12
Chi-square test	χ^2	0.864	39.744*		2.109		-		21.429*		13.830*	
	P	0.353	0.01		0.139		-		0.01		0.01	

Table 11: Comparison of serum ferritin levels between non-infected and infected children

	Normal		Moderate decrease		Mild decrease		Total	
	No.	%	No.	%	No.	%	No.	%
Non infected (n= 104)	104	100	0	0	0	0	104	100
Infected (n= 96)	83	86.5	4	4.2	9	9.4	96	100

Mild decrease in s. ferritin level = 10 – 20 ng/ml, Moderate decrease in s. ferritin level <10 ng/ml

Table 12: Effect of different intestinal parasites on serum ferritin level

Detected intestinal parasites	Normal		Moderate decrease		Mild decrease	
	No.	%	No.	%	No.	%
E. histolytica /dispar (n= 56)	54	96.4	0	0.0	2	3.6
G. lamblia (n= 8)	2	25.0	3	37.5	3	37.5
S. mansoni (n = 6)	5	83.3	0	0.0	1	16.7
H. nana (n= 6)	6	100.0	0	0.0	0	0.0
E. vermicularis (n= 4)	4	100.0	0	0.0	0	0.0
Mixed infection (n=16)	12	75.0	1	6.25	3	18.8
Total (n = 96)	83	86.5	4	4.2	9	9.38
Chi-quasre test	χ^2	40.640*				
	P	0.001				

Discussion

Intestinal parasitic infections still constitute one of the major causes of public health problems in the world, particularly in developing countries (Ekpenyong, 2008). Geographic and socioeconomic conditions, climate, poverty, malnutrition, personal and community hygiene, population density, unavailability of drinking water as well as poor sanitary facilities are the factors affecting the prevalence rate (Bdir and Adwan, 2010).

The infections are more common and tend to be of high intensity among school age children ranged between 6-14 years (Sharma *et al*, 2004; Ekpenyong, 2008) which affect their physical development, school attendance and ability to learn (Jasti *et al*, 2007).

The present study investigated the impact of intestinal parasites on hematological parameters (complete blood count and serum ferritin level) of school-age children in both urban and rural communities in Gharbia Governorate, Egypt.

Forty eight (48%) percent of the children were identified as having one or more of the following parasites present in their stool samples: *E. histolytica/dispar, G. lamblia, S. mansoni, H. nana, E. vermicularis* and *A. lumbricoides* which agrteed with Zaglool *et al* (2011) in Saudi Arabia, Oliveira *et al* (2015) in Angola and Yanola *et al* (2018) in Turkey. This was higher than (Ibrahium, 2011 and Bayoumy *et al*, 2016; 2018) who reported that parasitic prevalence among Egyptian school children in El-Minia, El Wadi El Gadeed and El Beheira Governorates were 29.3% 39.1% & 38.3% respectively.

However, the present results were less than that reported in Upper Egypt by El-Masry *et al.* (2007) who reported parasitic infections among Egyptian school children in Tamouh and rural school students in Sohag Governorate villages were 60.2% & 88.5%, respectively. These differences could be attributed to the locations of the studies, the season of the year the study was carried out and the method of stool analysis.

The distributions of hematological indices according to sex revealed that anemia was more prevalent among females in contrary to similar study by Oluwafemi and Oguntibeju (2003) and Le *et al* (2007) who showed percentage of anemia was more in males than females. The overall prevalence rate of anemia among the children was high (55%) and were anemic (Hb <11.5gm/dl). This agreed

with Kulsum *et al.* (2013) who found that, out of 700 blood samples examined from studied Indian children 399 (57%) were anemic and also agreed with Adebara *et al.* (2011) who reported that, more than a third of the Nigerian school children had Hb levels less than 11 g/dl and higher than Le *et al* (2007) and Oliveira *et al* (2015) who found that, prevalence of anemia among school children in rural Vietnam and Angola was 25% & 21.6% respectively. Non child had severe anemia which agreed with Adewuyi *et al.* (1992) and Adebara *et al.* (2011). The mean haemoglobin value was higher than results obtained in studies (Akinkugbe, 1977; Kulsum *et al*, 2013).

In the present study there was relationship between anemia and parasitic infections especially in *G. lamblia*, *E. histolytica/dispar*, *S. mansoni* and *H. nana* which agreed with Hendrickse (1991) and Shanti and Ghosh (2004). Among the most prevalent protozoa found was *G. lamblia* which observed among 100% of anemia while 75% had microcytosis and hypochromia. This percentage was higher than that reported by Carrilho *et al* (2011) as they found that of 80 *G. lamblia* patients only 21 (26.3%) had anemia. Also, Sackey *et al* (2003) reported that 26.4% of the Ecuadorian children had iron deficiency anemia and those with *G. intestinalis* infection exhibited significantly reduced mean serum hemoglobin levels compared to parasite-free ones. Also, association of *Giardia* with reduced serum hemoglobin was not correlated with age.

G. lamblia infection depends on the pathogenesis, the host immune and the activated proteases (Ma'ayeh *et al*, 2017). The interaction of the intestinal tract with the protozoa is also an important factor, where among the adverse effects, probably stimulated by the infection are villous atrophy in various degrees in the small intestine, associated with inflammatory infiltrate and crypt hypertrophy; bile acids alterations and decreased the disacaridases activity (Crua, 2003). As a consequence of these mechanisms, patient has poor absorption of sugars, fats, vitamins A, D, E, K, B12, folic acid, iron and zinc (Rey, 2001). This poor absorption of iron described may explain the high percentage of anemia among individuals infected by *G. lamblia* in the present work. In the present work, the negative impact of giardiasis on child iron status agreed with Shubair *et al.* (2000) who linked giardiasis with significantly reduced mean hemoglobin level in Palestinian and Egyptian children.

Comparison of the haematological status between infected and non-infected children showed that the mean hemoglobin indices of the latter were higher. This agreed with Curtale *et al.* (2000) in Alexandria, Yentur Doni *et al.* (2014) in Turkey and Oliveira *et al.* (2015) in Angola. The association between low hemoglobin and parasitic positivity was possible because intestinal parasites were lodged in the duodenum and jejunum, the iron absorption site (Alfred *et al*, 2013).

The present work showed that lecocytosis (mainly eosinophilia) in infected cases as compared to non-infected persons. This agreed with the results of Yanola *et al.* (2018) who showed increase number of W.B.Cs (10.6) $x10^3$ cell/ mm^3 in infected cases in comparison with control (6.31) x 10^3 cell/mm^3 (74.7%). This may be due to the eosinophils ability to destroy parasites by attaching to its wall and secretion of granules leading to their destruction. Low 6.5% of children having low serum ferritin level indicated iron deficiency anemia among them. Iron store of studied population was adequate with only 6.5% had exhausted iron stores. Serum ferritin increased during infection, giving false negative results for iron deficiency (Lipschitz et *al*, 1974). A higher cut-off value for serum ferritin to determine iron deficiency in infected populations and/or inflammatory diseases was highly prevalent (Punnonen and Irjala, 1997).

In the present study, there was a significant association between serum ferritin values

and parasitosis. Six *G. lamblia* cases had low serum ferritin level and exhausted iron stores. This was at variance with studies done in other parts of Africa (Stoltzfus *et al*, 1997; Olsen et *al*, 1998) where the exhausted iron stores degree was higher. This could be attributed to low hookworm cases in the present study. Adebara *et al.* (2011) found association between intestinal helminthiasis and serum ferritin level among Nigerian school children and reported that ferritin levels were lower in hookworm children. Also, 36% of hookworm pupils had exhausted iron stores. But, neither *A. lumbricoides* nor *T. trichiura* was related to any iron deficiency.

Conclusion

The high prevalence of intestinal parasitic infection and anemia observed in this study calls for a comprehensive public health intervention. There is a strong need for helminthes control as a strategy to control iron deficiency in the school children where anthelminthic drugs are safe and cheap and giving as periodic delivery to them.

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