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INTESTINAL PARASITIC INFECTION IN EGYPTIAN CHILDREN: COULD IT BE A RISK FACTOR FOR IRON DEFICIENCY ANEMIA?

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Abstract

A case control study that associates IDA with different parasitic infections and socioeconomic factors. The study enrolled 194 children with IDA and 180 age matched control. Patients diagnosed as IDA by complete blood count (CBC), and iron indices. All cases were subjected to complete history, anthropometric measures, and CBC and stool analysis. *Ancylostoma duodenal*, *Ascaris lumbercoides* and *Giardia lamblia* infections, lower family income, increased number of family members and eating meat in low frequency could be independent risk factors for IDA as detected by multivariate regression analysis. *A. duodenale* and *G. lamblia* were associated with lower ferritin levels in anemic patients. No significant associations as regards residence, body mass index, mother employment or education levels (p>0.05).
Keywords: Iron deficiency anemia, Parasites, Socioeconomic.

Introduction

Iron (Fe) deficiency anemia (IDA) is a significant public health problem as it is the most common cause of anemia all over the world. About 40-50% of children are iron deficient in developing countries (UNICEF, 2002). In Egypt, the prevalence rates of IDA were 43% of children aged 6-24 months (Elalfy *et al,* 2012) and 25.6% of those aged 6-11years (Abdel-Rasoul *et al,* 2015). Iron is an important element for brain growth and development especially during infancy as it is required for several functions in the brain including nerve fiber myelination and acting as a cofactor in several reactions for neurotransmitter synthesis accordingly cognitive dysfunction is a major consequence of iron deficiency (Rao and Georgieff, 2001). Reduced work capacity is additional consequence of iron deficiency (Haas and Brownlie, 2001). IDA in African children has numerous risk factors which illustrates the complex etiology (WHO, 2008). Inadequate nutritional iron intakes, insufficient absorption of iron, and iron loss by bleeding are the main causes of IDA. Bleeding may be via intestinal, urinary tract or uterine route. Parasitic infestation may be a cause iron-deficiency anemia in children as a result of mal absorption and intestinal bleeding caused by the worm, which may be non-noticeable in feces (Calis, 2008).

Parasitic infections are considered a major public health problem especially in developing countries. Globally intestinal parasitic infections were predicted in about 3.5 billion people, the great majority were children (Brooker *et al*, 2009). Parasitosis resulted in poor growth, physical weakness, decrease in educational performance and other morbidities (Nokes, 1994). Intestinal parasites such as amoebiasis, ancylotsomiasis, trichiuriasis and ascariasis were considered the commonest distributed parasites worldwide (WHO, 1987). The main causes of iron deficiency anemia should be investigated using various interventions that based on clinical and epidemiological information.

This work aimed to study the effect of common intestinal parasitosis and triggering iron deficiency anemia, in relation to socio-demographic and economic factors of children attending Al-Fayoum University Pediatrics Hospital.

Subjects, Materials and Methods

Study design and setting: A case control study was carried out on 194 patients with IDA and 180 ages matched healthy controls at the time from May2016 to September 2016 in outpatient clinic of Al-Fayoum University Pediatrics Hospital. Inclusion criteria were children aged 2 to 12 years old diagnosed as IDA by CBC, serum ferritin, serum iron, total iron binding capacity. Control group included cross ma-tched children with normal CBC (normal he-moglobin and other hematological indices). Exclusion criteria were children less than 2 years, more than 12 years with associated chronic disease,

All cases and controls were subjected to full history taking including age, sex, residence, mother education and employment, family members per house, family income, and average frequency of eating meat per week. Weight and heights were obtained using appropriate calibrated devices, and then BMIs were calculated

Laboratory examination: Aseptic venous blood samples were collected and examined for hematocrit value, hemoglobin concentration, erythrocyte indices as mean corpuscular Hb (MCH), MCH concentration (MCHC), corpuscular volume (MCV), and red cell diameter width (RDW). The complete blood count (CBC) was done using Coulter 1660 to determine the MCH, MCV and RDW. Anemia was identified by Hb concentration below the adjusted values for age groups (Wonke *et al*, 2007). Microcytosis and hypochromia were diagnosed when the MCV was below 80 fl and MCH below 27 pg. (Johnson-Wimbley, 2011).

Anemic children were subjected to: Serum ferritin measure using Immulite/Immulite 1000 ferritin kits (Siemens, Los Angeles, California), serum iron levels, total iron binding capacity (TIBC) using calorimetric kits (Stanibo Co., USA). Transferrin saturation was calculated as serum iron divided by TIBC. Iron diagnostic for IDA consisted of a low Hb for age, together a low SF with below 12μg/L or low TS below 16% (Johnson-Wimbley, 2011). Hb electropho-resis was done to exclude β thalassemia minor (Eden and Sandoval, 2012). .

Fecal samples were collected in dry plastic labeled container and examined macroscopically for color, consistency, pin warms, larvae, blood and mucous. Direct wet smear methods using iodine and lacto-phenol cotton blue and concentration technique were used to examine fecal sample (El-Naggar *et al,* 2006). Formalin-ethyl acetate sedimentation was done and examined by direct wet smear using Modified Ziehl-Neelsen stain for cryptosporidiosis (Garcia, 2007).

Ethical consideration: Protocol was approved by the ethical committee of Faculty of Medicine, Al-Fayoum University. Informed oral consent was obtained from each child's parents before participation in the study.

Statistical analysis: Data was collected and coded to facilitate data manipulation and double entered into Microsoft Access and data analysis was performed using SPSS software version 18 under windows 7. Simple descriptive analysis in the form of numbers and percentages for qualitative data, and arithmetic means as central tendency measurement, standard deviations as measure of dispersion for quantitative parametric data, and inferential statistic test: Independed student t-Test used to compare measures of two independent groups of quantitative data. One way ANOVA test was used to compare more than two independent groups of quantitative data and Chi square test to compare two of more than two qualitative groups. Multiple linear regressions tested association between quantitative dependent and in-dependent variables and detection of risk factors. The $P \leq 0.05$ was considered cut-off value for significance.

Results

The study included 194 patients with iron deficiency anemia. Mean age of study patients was (6.5±3.5years), the mean HB level was (8.3±1.2g/dl). Mean MCV was (67.7±8.4fL), mean MCH was (23.4±3.6pg) and mean RDW was (18.36 ±1.27). Mean serum ferritin level was (10.93±2.78μg/l) and mean TIBC was (405.5±55.6μg/dL). 55.7% of patients are resident in rural area and 44.3% were from urban areas. As regards mother's employment level around

69.1% of mothers were non workers. Prevalence of parasitic infection was 17.5%, 33%, 38.1% & 18% with hookworm, *A. lumbricoides*, *E. vermicularis* and *H. nana* respectively. *Giardia lamblia* and *E. histolytica* was found in 25.8% & 33% of patients respectively.

Infection with *A. duodenale, A. lumbricoides* and *G. lamblia* were significantly associated with IDA as they were presented with higher frequency in anemic patients. No significant differences between anemic and control as regards infection with *H. nana, E. vermecularis* or *E. histolytica*. Intestinal infection with *A. duodenale* and *G. lamblia* were significantly associated with lower ferritin levels in anemic patients (P-value 0.01 & 0.03 respectively), without significant associations with serum ferritin as regards other parasites. Mean ages were 6.6±3.5years & 7.4±3.2 for patients and control respectively without significance differences (P= 0.15). No differences were found between patients and controls regarding sex, residence or mother work. Monthly income less than 1200 LE was found in higher percentage among patients with iron deficiency anemia (p. value= 0.000).

There was a significant difference between patients and controls as regards number of family members/house, family size less than 5 was associated with less frequent IDA compared with those with more than 5 members (P= 0.001). There was also a significant difference between patients and control as regards Frequency of eating animal meat per week, eating meat less than 2 times per week was significantly associated with more frequent IDA(P = 0.000). No significance differences between patients and control was found as regards level of mother education or BMI.

The multivariate linear regression model analysis illustrated significance predictors with p-value <0.05 to family income, frequency of eating meat, number of family members/house (P= 0.00, 0.00 & 0.002 respectively), infection with *A. lumbricoides*, *A. duodenale* and/or *G. lamblia* were significance predictors (P= 0.004, 0.017 and 0.014 respectively). Details were given in tables (1, 2, 3, 4, 5 & 6).

Table 1: Clinical and socio-demographic characters of anemic patients.

Variables	Number (n=194)	%
Male	110	56.7%
Female	84	43.3%
Body mass index Percentile: $>3^{rd}<95^{TH}$	114	58.7%
Below 3^{rd}	62	32%
Above 95th	18	9.3%
Residence:Rural	108	55.7%
Urban	86	44.3%
Mother education level Illiterate	48	24.7%
Primary	67	34.6%
Middle level	58	29.9%
High education	21	10.8%
Mother employment Worker	60	30.9%
Non worker	134	69.1%
Family members: ≤ 5	76	39.2%
> 5	118	60.8%
Parasitic infestation		
Hookworm	34	17.5%
Ascaris lumbercoides	64	33%
Enterobius vermicularis	74	38.1%
Hymenolepis nana	35	18%
Giardia lamblia	50	25.8%
Entameba histolytica	64	33%

Table 2: Comparison between patients and control as regards intestinal parasitic infection

Variable	Mean hb±SD(g/dl)	Frequency in 194 patients (%)	Frequency in 180 control (%)	P. value
Hook worm +ve Hook worm -ve	8.6±2.3 10.6±2.4	34(17.5) 160(82.5)	10(5.6) 170(94.4)	0.000
Ascaris +ve *Ascaris* -ve	9.6±2.4 10.7±2.5	64(33) 130(67)	32(17.8) 148(82.2)	0.001
Enterobius +ve *Enterobius* -ve	10.3±2.6 10.5±2.4	74(38.1) 120(61.9)	68(37.8) 112(62.2)	0.5
H. nana +ve *H. nana* -ve	9.9±2.3 10.5±2.5	35(18) 159(82)	22(12.2) 158(87.8)	0.077
Giardia +ve *Giardia* -ve	9.7±2.2 10.6±2.4	50(25.8) 144(74.2)	24(13.3) 156(86.7)	0.002
Entameba +ve *Entameba* -ve	10.5±2.5 10.4±2.4	64(33) 130(67)	64(35.6) 116(64.4)	0.34

Table 3: Effect of parasites on ferritin level in patients with iron deficiency anemia

Variable		Mean ferritin±SD	F	P. value
Hook worm	Yes	9.83±2.82	6.59	**0.01**
	No	11.16±2.72		
A. lumcricoides	Yes	10.62±3.23	1.23	0.27
	No	11.08±2.52		
E. vermicularis	Yes	10.62±2.57	1.5	0.23
	No	11.12±2.89		
H. nana	Yes	10.43±2.48	1.5	0.21
	No	11.1±2.8		
G. lamblia	Yes	10.2±2.5	4.6	**0.032**
	No	11.2 ±2.8		
E. histolytica	Yes	10.8±2.35	0.21	0.64
	No	11.00±2.97		

Table 4: Comparison of socio demographic parameters between IDA patients and control

Variable		Frequency in 194 patients (%)	Frequency in 180 control (%)	P. value
Sex	Male	110(56.7)	105(85.3)	0.45
	Female	84(43.3)	75(41.7)	
Residence	Rural	108(55.7)	93(51.7)	0.21
	Urban	86(44.3)	87(48.3)	
Mother work	Yes	60(30.9)	68(37.8)	0.2
	No	134(69.1)	112(62.2)	
Income	>1200LE	74(38.1)	104(57.8)	0.000
	<1200LE	120(61.9)	76(42.2)	

Table 5: Multiple comparisons of some socio-demographic parameters between IDA patients and control

Variable	Multiple comparison	95% Confidence Interval	P. value	F	P. value
Family members/ house	≤ 5versus>5<7	0.046, 0.262	0.005	7.37	0.001
	≤ 5versus≥7	0.120, 0.422	0.000		
	>5<7 versus≥7	-0.036, 0.275	0.13		
Consumption of meat/week	≤2 versus>2<5	-0.360, -0.150	0.000	15.12	0.000
	≤2versus≥5	-0.499, -0.162	0.000		
	>2<5versus≥5	-0.249, 0.098	0.39		
Level of mother education	Illiterate vs. 1ry school	-0.186, 0.102	0.56	2.38	0.069
	Illiterate vs. 2ry school	-0.292, 0.006	0.04		
	Illiterate vs. University	-0.358, -0.006	0.043		
Body mass index	Normal vs. underweight	0.004, 237	0.04	2.25	0.11
	Normal vs. overweight	-0.185, 0.158	0.88		
	Underweight vs. over	-0.320, 0.052	0.158		

Discussion

Investigating the effect of intestinal parasitic infection as a cause of IDA was necessary to help in directing interventions to face this problem especially in parasites endemic areas (Pullan and Brooker, 2008). The study revealed that *Ancylostoma* was a significant independent risk factor for IDA (P= 0.017), associated with decreasing levels of ferritin in anemic children (p= 0.01). In concordance, association of IDA with helminthic parasite was documented (Stoltzfus, 1997; Crompton, 2000). *Ancylostoma* is one of the most important parasites that infect human (Stephenson, 1989). Iron deficiency anemia caused as a result of consumption of blood by *Ancylostoma* and coagulase released led to chronic intestinal blood loss (Ganz, 2006). Growing children have relatively poor iron stores and so they are more liable to IDA due to blood loss. The intensity of infection and actual Iron stores levels could affect the Progression to parasite induced iron deficiency anemia. Treatment of hookworm infection caused enhancement in iron status and improvement in work capacity (Stephenson, 1989).

Table 6: Regression analysis to determine risk factors for IDA.

Factors	OR	95.0% Confidence Interval for OR		Sig. (P. value)
		Lower Bound	Upper Bound	
(Constant)	0.133	-0.458-	0.724	0.659
Age	0.013	-0.001-	0.028	0.065
Sex	-0.067-	-0.167-	0.034	0.191
BMI	-0.039-	-0.110-	0.032	0.279
rural	-0.001-	-0.099-	0.097	0.986
Mother work	-0.028-	-0.133-	0.077	0.596
Mother education	0.037	-0.015-	0.089	0.161
Income	0.189	0.094	0.284	0.000
Meat/week	0.151	0.077	0.224	0.000
Family members	-0.107-	-0.176-	-0.039-	0.002
A. lumcricoides	0.159	0.050	0.269	0.004
E. vermicularis	0.057	-0.042-	0.156	0.261
A. duodenale	0.188	0.034	0.341	0.017
H. nana	0.004	-0.129-	0.137	0.956
G. lamblia	0.149	0.030	0.269	0.014
E. histolytica	0.021	-0.083-	0.125	0.692

This study revealed that *Ascaris* was another significant predictor for IDA. This agreed with Osazuwa *et al.* (2011) and Ngui *et al.* (2012) who found significant association of *Ascaris* infection and anemia. It impairs the nutritional status of infected children and adolescences by increasing nutrient demands of the warm, anorexia and by blockage of the mucosa absorbing surface; iron absorption takes place in duodenum in infected patient (Jardim-Botelho *et al,* 2008). Also, *Ascaris* can cause Vitamin A mal absorption and lactose intolerance that affect children growth (Bethony *et al,* 2006). Treatment of *Ascaris* improved IDA (García-Leiva *et al,* 2008). But, Islek *et al.* (1993) didn't find associations between anemia and *Ascaris.*

The prevalence of *G. intestinalis* in Egypt was 11% (el-Naggar *et al*, 2006), which showed that giardiasis had a significant independent effect on IDA. Giardiasis was associated with IDA, diminished iron absorption and decreased iron indices in infected (Abou-Shady *et al,* 2011; Ertan *et al,* 2002; Hussein *et al,* 2016). Its treatment improved hemoglobin level and all iron indices in iron deficiency anemia (Monajemzadeh, 2008; Prado *et al,* 2005). But, Al-Mekhlafi *et al.* (2005) found that treatment of asymptomatic giardiasis didn't affect hematological nor iron indices in children with IDA.

In the present study, age was not associated with IDA. This did not go with Habib *et al*. (2016) who found that anemia was associated with younger age due to iron deficiency. Infant and toddlers aged 13 to 36 years were more exposed to severe IDA than younger ones (Paoletti *et al,* 2014). Absence of the effect of age may be due to exclusion of infants less than 2 years in this study. Increased prevalence of intestinal parasites in older aged children could increase the possibility of IDA (Chang Cojulun *et al,* 2015).

In this study, low monthly income (<1200 LE) and eating meat less than twice weekly were risk factors for IDA as revealed via regression analysis. This agreed with Danquah *et al*. (2014) who found low house income was independently associated with low hemoglobin level. The low income limited access to foods rich in iron. Plant based diets (no meat and high phytic acids) in families with low income have lower level of iron bioavailability, and people following these diets are more prone to iron defeciency (National Health and Medical Research Council, 2014). Brussard *et al.* (1997) found a negative association between iron status and consuming vegetable protein. High number of family members was also independently associated with reduced hemoglobin level and IDA as revealed by regression analysis. Lower intake of iron in crowded families along with a higher rate of exposure to infections and intestinal helminthic infestations may be reasonable explanations (Curtale *et al,* 2000).

On the other hand, Abo-Madyan *et al*. (2004) Ezbet El-Bakly (Tamyia Center) Al-Fayoum Governorate carried out a field survey to assess the efficacy and safety of Mirazid in the treatment of human fascioliasis. Among 1019 individuals examined for parasitosis, the prevalence of fascioliasis was 1.7% and the geometric mean egg count was 33.2 eggs/gram stools. About 23.5% of patients were asymptomatic. The most frequent symptoms were abdominal dis\tension and flatulence (76.5%), right hypochondrial pain (17.6%) and epigastric pain (17.6%). igns were pallor (52.9%), tender right hypochondrium (23.5%) and tinge of jaundice (17.6%). Others were *S. mansoni* (2.4%), *E. vermicularis* (2%), *A. lumbricoides* (1.7%), *H. nana* (1.7%), *Taenia saginata* (0.3%).

Apart from Al-Fayoum, Morsy *et al.* (1991) in Qualyob City, Qualyobia Governorate examined two primary schools of 486 school children (6-12 years old) had pediculosis (16.04), schistosomiasis (8.8%), *E. histolytica* (7.81%), *G. lamblia* (9.05%), *T. saginata* (0.41%), *A. lumbricoides* (9.05%), *E. vermicularis* (0.9%) & *H. nana* (9.87%). The lice was 17.8% of total parasites detected. Autoinfection parasites were 43.02%, those by skin penetration were 9.84%, those by meat consumption were 0.45% and by other modes were 28.8%. El-Shazly *et al.* (2006) in Dakahlia Governorate reported intestinal helminthes in a descending order of abundance were *S. mansoni* (5.3%), *Fasciola* sp. (4.8%), *H. heterophyes* (4.2%), *H. nana* (3.9%), *Trichostrongylus* sp. (2.6%), *A. lumbricoides* (1.8%), *Strongyloides stercoralis* (1.5%), *H. diminuta* (1.4%), *Taenia saginata* (1.1*%), E. vermicularis* (by smear; 1.1 %), *T. trichura* (0.7%) and *A. duodenale* (0.1%). Intestinal protozoa in an abundance descending order were *Blastocystis hominis* (22.4%), *G. lamblia* (19.6%), *E. histolytica/E.dispar* (19%), *Iodamoeba butschlii* (16%), *Cryptosporidium parvum* (14.3%), *E. coli* (9.7%), *Isospora hominis* (7.7%), *Endolimax nana* (6.9%), *E. hartmani* (5.9%), *Dientamoeba fragilis* (5.1%), *Chilomastix mesnili* (5.1%), *Trichomonas hominis* (4.2%), *Cyclospora cayetanensis* (4.2%), *Microsporidia* spores (3.2%), *Enteromonas hominis* (1.9%) and *Embadomonas intestinalis* (1.3%). Eraky *et al*. (2014) in Banha City in stool examination for intestinal parasites found in 157/530 (29.6%) samples. *G. lamblia* cysts were the commonest one (8.8%) followed by *Entamoeba* spp. cysts (6.8%), *E. vermicularis* (4.9%), helminth larvae (3.6%), *H. nana* (2.8%), *H. diminuta* (2.1%), & *A. lumbricoides* (0.6%). Contaminated vegetable was lettuce (46%), watercress (41.3%), parsley (34.3%), green onion (16.5%), and leek (10.7%). These results indicate a significant seasonal variation ($P < 0.05$), with high prevalence in summer (49%) and low in winter (10.8%). Shehata and Hassanein (2015) in Alexandria reported 87/200 (43.5%) mentally handicapped persons with parasitosis; 44.6% and 42.6% for non-institutionalized and institutionalized ones, respectively. They were 46.7% & 38.5% males and females. Commonest were *Cryptosporidium* sp. (23.5%), *Microsporidia* (15%), *G. lamblia* (8.5%), *D. fragilis* (8%), *C. cyatanensis* (7.5%), *B. hominis* (6.5%), *E. histolytica* (5.5%) and *E. coli* (2.5%). *I. belli* and *E. vermicularis* were 1.5% and *Iodamoeba butschlii* was (1.0%).

Conclusion

The data highlights the importance of *Ancylostoma, Ascaris* and *Giardia* infections as risk factors for IDA among children. *Ancylostoma* and *Giardia* were associated with lower levels of ferritin in anemic children. Low family income, low frequency of eating meat and higher family size were additional important contributors for IDA. This would direct implementation of integrated health programs in areas of parasitosis to improve hygienic and public health aware-ness. Screening programs for parasites is a must. Nutrition education with iron rich supplement must be recommended in high risk groups. Health sectors must take an active role in improving healthy public policies to alleviate diseases related to poverty.

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