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UNDERWEIGHT AS A RISK FACTOR FOR MULTIPARASITISM AND ITS CLINICAL SIGNIFICANCE AMONG EGYPTIAN CHILDREN

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Abstract

Although multiparasitism is a common phenomenon, but its impact on young children nutritional and developmental status is underestimated. This cross-sectional study aimed to statistically correlate single/multiple parasitic infections detected with underweight, demographic and clinical data. Stool samples were collected from 222 Egyptian children from 1 to 5 years old who were attending the Diagnostic and Research Unit of Parasitic Diseases. Stool samples were screened for parasitic species using light microscopy of direct, trichrome and modified kinyoun stained smears.

Nutritional status was evaluated using weight-for-age plotting against standard reference growth charts. Questionnaire was used to collect socio-demographic and clinical data of children under study. The overall prevalence of parasitosis was 51.8%. Among infected children, polyparasitism was significantly higher among underweight (66.7%; P<0.001), and in males compared to females (31.6% vs. 16.7%; P =0.011). Univariate analysis showed significant associations between underweight and *Giardia lamblia* co-infection with other species (P < 0.001). Multiparasitism should be of more concern especially to this age group, as it can exacerbate children's morbidity. However, several methodological challenges remain when it comes to detecting parasite interactions in resource-constrained settings.

Key words: Children, Multiparasitism, Significance, Underweight

Introduction

Parasitic diseases in tropical and subtropical areas play an important role in morbidity and mortality in these areas. One-third of all deaths are due to infectious viral, bacterial and parasitic diseases according to a recent World Health Organization (WHO) "global and regional causes of death" report (WHO, 2018). Current reports showed that about 2 billion people are established cases of parasitic infections with millions are still at risk of occurrence of some kind of morbidity (Hotez *et al*, 2008; Pullan and Brooker, 2012). Severity increases according to intensity, number and type of incriminated species of parasitic infestation (McKenzie, 2005; Steinmann *et al*, 2010). Parasitic species co-infections can have chronic diverse consequences on host health especially children's nutrition and development (Hall *et al*, 2008; Oninla *et al*, 2010). The closed circle of nutritional impairment as evidenced by the growth retardation, immune dysfunction and increased susceptibility to intestinal mono or poly-parasitism, which subsequently will compete for host nutrients, has been illustrated in variety of cross-sectional studies (Cox, 2001; Ordonez and Angulo, 2002; Casapía *et al*, 2006; Jardim-Botelho *et al*, 2008). Although detection of multiple parasites infection within the same individual is common among the routine laboratory work (Steinmann *et al*, 2008), only few analyzed data is present to assess the extent of morbidity and risk factors associated with polyparasitism among populations in low and middle income settings (Sayasone *et al*, 2015).

The current study was a cross-sec-tional survey on Egyptian Children aged between 1 to 5 years using personal questionnaire, parasitological and clinical examination to objectively assess the relationship between single or multiple parasitic infections with weight loss, demographic and clinical parameters.

Material and Methods

A cross-sectional study was conducted in Diagnostic and Research Unit of Parasitic Diseases (DRUP), Kasr Al Ainy, Cairo University, Cairo, Egypt on children from one to five years old, who were referred to the unit for stool analysis. A stool sample was collected from each child fulfilling criteria for our study from July 2016 to December 2017.

Children from 1 to 5 years old who did not receive any antibiotics, anti-parasitic medications, vitamins or dietary supplements within month preceding were included; but cases with known chronic diseases were excluded

Interview questionnaire: Children data was collected from guardians of children under study, and through personal communication with the responsible pediatrician. Data entitled demographic, breast-feeding and clinical data as, GIT symptoms, and general condition, appetite loss and weight.

Undernutrition assessment: The standard reference growth charts approved by Endocrinology and Metabolic Pediatric unit (EMPU) and National Research Center (NRC), in collaboration with Life Span Health Research Center, Wright-State University (Ghalli *et al,* 2008) was used to evaluate weight status and under-nutrition in children under study.

Stool samples were collected in a clean-labeled plastic container with tight lid. Contaminated samples with water or urine were rejected. The samples were initially examined microscopically using direct saline and iodine wet mount under low (10´) power and (40´) high power objectives. Direct examination by saline wet mounts was made by mixing a small volume of pellet with a drop of physiological saline on a glass microscope slide. Iodine wet mounts were prepared by adding pellet to a drop of Lugol's iodine. The rest of the sample was re-examined after concentration using Modified Ritchie's biphasic method (Garcia, 2007). A portion of the specimen was used to prepare thin smears for permanent staining by trichrome stain for intestinal protozoa and modified Kinyoun staining for *Cryptosporidium* according to manufacturer instructions (Eng Scientific Inc., N.J. 07012-1708, USA). Multiple slides were prepared from each stool sample.

Ethical considerations: Verbal consent was obtained from the children parents or guardians after explanation to them the aim of the study. All patient data was confidential and anonymous.

Statistical analysis: Data was coded and entered using Microsoft office-excel 2010. Statistical analysis was done using IBM SPSS version 24 (IBM Corporation, USA, Armonk, New York). Frequencies (number) and relative frequencies (percent) were used to summarize qualitative variables while mean, median; quartiles and standard deviations were used for quantitative variables. Comparison between groups was done using chi-square test. P value less than or equal to 0.05 was considered significant.

Results

A total of 222 Egyptian children, 114 (51.4%) males and 108 (48.6%) females, participated in the study. Their age ranged from 1 to 5 years with mean and standard deviation of 3±1.37 years. More than three fourths of them were living in urban areas, whose houses were concrete-built with electricity, fresh tap water supply and modern-based toilet facilities. Breast-feeding 201 (90.5%) was the predominant nourishment way.

Children with clinical manifestations were 182(82%), while the rest were referred for routine lab. One or more of these clinical presentations were recorded upon the physician visit; diarrhea, abdominal pain or flatulence, and vomiting (Tab.1). Weight measurements as a nutritional status indicator showed a range from 5 to 23 kilograms with mean and standard deviation of 12.5±3.78 kg. According to weight-for-age scales, 75 (33.8%) children were underweight (z-score ≤ 2).

Table 1: General characteristics of participating children

Variants	Frequency
Male	114 (51.4%)
Female	108 (48.6%)
Feeding	
Breast feeding	201 (90.5%)
Bottle feeding	21 (9.5%)
Residence	
Urban	178 (80.2%)
Rural	44 (19.8%)
Gastrointestinal complaints	
Diarrhea	120 (54.1%)
Vomiting	58 (26.1%)
Pain	115 (51.8%)

Stool samples showed parasites in 115/222 (51.8%). They were *G. lamblia*, *E. histolytica/dispar* complex, *C. parvum* and *B. hominis,* respectively (Fig. 1). Fifty-four (47%) of infected samples had more than one parasite, 35(31%) showed two parasites per, 18(16%) showed three parasites and one child had 4 parasites (0.9%). The commonest was *G. lamblia* co-infection with other parasites (Tab. 2) in comparison to other single and multiple infections (P< 0.001).

Table 2: Frequency profile of single/multiple species co-infection

variants	Frequency
Single infection	
Giardia lamblia	40(18%)
Cryptosporidium parvum	12(5.4%)
Hymenolepis nana	4(1.8)
Entamoeba coli	2(0.9%)
Blastocystis hominis	1(0.5%)
Dientameoba fragilis	1(0.5%)
2 parasites co-infection	
G. lamblia and *E. histolytica/dispar* complex	15(6.8%)
G. lamblia and *B. hominis*	4(1.8)
G. lamblia and *C. parvum*	4(1.8)
G. lamblia and *E. coli*	4(1.8)
G. lamblia and *Endolimax nana*	3(1.4%)
E. histolytica/dispar complex and *C. parvum*	2(0.9%)
G. lamblia and *H. nana*	1(0.5%)
C. parvum and *B. hominis*	1(0.5%)
C. parvum and *H. nana*	1(0.5%)
3 parasites co-infection	
G. lamblia, *E. histolytica/dispar* complex and *B. hominis*	6(2.7%)
G. lamblia, *E. histolytica/dispar* complex and *C. parvum*	4(1.8)
G. lamblia, *E. histolytica/dispar* complex and *E. coli*	4(1.8)
G. lamblia, *E. histolytica/dispar* complex and *Iodameoba butschlii*	1(0.5%)
G. lamblia, *H. nana* and *Dientameoba fragilis*	1(0.5%)
G. lamblia, *C. parvum* and *B. hominis*	1(0.5%)
G. lamblia, *H. nana* and *B. hominis*	1(0.5%)
4 parasites co-infection	
G. lamblia, *E. histolytica/dispar* complex, *C. parvum* and *E. coli*	1(0.5%)

Males showed higher prevalence of *E. histolytica/E. dispar* complex 24 (68.6%), *G. lamblia* 55(61.1%) and more than one parasite 36(31.6%), males infection was than in females (P <0.05). A positive association was between multiparasitism and rural areas, bottle-feeding and suffered from diarrhea as compared to non-infected ones (Tab. 3).

Table 3: Demographic and clinical parameters in relation to multi-parasitic infections

variants	Not-infected		One parasite		> One parasite		P value
	No.	%	No.	%	No.	%	
Male	45	39.5	33	28.9	36	31.6	0.011*
Female	62	57.4	28	25.9	18	16.7	
Residence							
Urban	88	49.4	52	29.2	38	21.3	0.103*
Rural	19	43.2	9	20.5	16	36.4	
Breast feeding	104	51.7	56	27.9	41	20.4	<0.001*
Bottle feeding	3	14.3	5	23.8	13	61.9	
Diarrhea							
Yes	49	40.8%	31	25.8%	40	33.3%	0.003*
No	58	56.9%	30	29.4%	14	13.7%	
Vomiting							
Yes	23	39.7%	18	31.0%	17	29.3%	0.308
No	84	51.2%	43	26.2%	37	22.6%	
Pain							
Yes	69	60.0%	23	20.0%	23	20.0%	0.001*
No	38	35.5%	38	35.5%	31	29.0%	

* Significant association (P < 0.05)

Children living in rural areas were significantly highly underweight. They were significantly bottle-fed compared to breast-feed. A positive association was between infection and underweight (P=0.004); 49 (65.3%) from infected children were underweight (z-score ≤ 2). *G. lamblia* and *E. histolytica/dispar* complex were significantly (P<0.001) associated with low weight-for-age (z-score ≤ 2) (Tab. 4). Children with multiparasites were significantly underweight than children with no or single infection, P value <0.001 (Fig. 2). A significant association between underweight and existence of "*Giardia* and other parasites" in the same sample was detected (P < 0.001).

Table 4: Chi-square analysis of underweight (z-score ≤ 2) with different risk predictors

variants	Yes		No		P value
	No.	%	No.	%	
Male	44	38.6	70	61.4	0.119
Female	31	28.7	77	71.3	
Breast feeding	60	29.9	141	70.1	<0.001*
Bottle feeding	15	71.4	6	28.6	
Residence Urban	54	30.3	124	69.7	0.029*
Residence Rural	21	47.7	23	52.3	
Diarrhea					
Yes	48	40.0	72	60.0	0.034*
No	27	26.5	75	73.5	
Parasitic infection					
No	26	24.3%	81	75.7%	0.004*
Yes	49	42.6%	66	57.4%	
Single/Multiple parasite					
One parasites	13	21.3%	48	78.7%	<0.001*
> One parasite	36	66.7%	18	33.3%	
Giardia lamblia					
No	33	25.0	99	75.0	0.001*
Yes	42	46.7	48	53.3	
Entamoeba histolytica/dispar complex					
No	47	25.1	140	74.9	<0.001*
Yes	28	80.0	7	20.0	

* Significant association (P < 0.05)

Discussion

Parasitosis form the global health burden in many developing countries worldwide including Egypt, with their morbidity predictors not fully resolved (El-Nadi *et al*, 2017). The present results showed that 51.8% of children have got at least one parasitic species. This finding was in accordance with a recent Egyptian study conducted in Assiut governorate, which reported 55.7% of parasitic infections among children from 1-6 years old (Yones *et al*, 2015).

Giardia lamblia was the predominant parasite (41 %) among infected children. Many studies had proven the prevailing presence of *Giardia* in young children, compared to older ones (Keiser *et al*, 2002; Nguhiu *et al*, 2009). Diverse isolates and inconsistent host immune response play an important role in the discrepant *Giardia* infection patterns (Majewska, 1994).

The current study revealed that multiparasitism is an important finding among Egyptian children; by which 47% of the infected children harbored 2 or more parasites concurrently. This finding is in agreement with those of previous reports and studies which proved that polyparasitism was a common model for parasitic existence rather than a single parasite presentation (Brooker *et al*, 2000; Keiser *et al*, 2002; Nguhiu *et al*, 2009).

In spite the fact that *G. lamblia* is resistant for any concomitant species, due to damage of intestinal mucosa and bacterial overgrowth, which render it, not suitable for other parasites to colonize (Blackwell *et al*, 2013), a significant association with *E. histolytica/E. dispar* complex and *Blastocystis hominis* was discovered in our study. *Giardia lamblia* co-infection with other parasites showed significance with low weight-for-age in comparison with *Giardia* infection alone.

This finding corroborates well with that in a previous report (Carvalho-Costa *et al*, 2007), a finding that necessitate more research to explain.

Males showed significantly higher prevalence in contracting *E. histolytica/E. dispar* complex, *G. lamblia* and hosting “more than one” parasite than females in our study group. This was confirmed with previous data (Nguhiu *et al*, 2009), but contrasts with (Weerakoon *et al*, 2018), who detected no significant gender associations. The impact of sex-related hormones on the host immune response towards infectious agents, specifically parasites, was previously acknowledged (Sevilimedu *et al*, 2016).

The authors reported significant association of underweight among infected children. Furthermore, the prevalence of underweight was obviously higher among children infected with more than one parasitic species (48%) than in children with only one parasite (17.3%). These results were in line with other surveys done in low-middle-income setting, which proved the aggravation of nutritional deficiency in multiparasitism children compared to single parasite cases in Gaza (Shubair *et al*, 2000), Mexico (Quihui-Cota *et al*, 2004), Tehran (Nematian *et al*, 2008), Nigeria (Opara *et al*, 2012) and Ethiopia (Degarege and Erko, 2013). Observing a significant increase in weight-for-age values after follow-up with anti-parasitic treatment proved these findings further (Taylor-Robinson *et al*, 2012; Degarege and Erko, 2013). This significant positive and strong correlation points could be explained by either diminished nutritional absorption, damaged intestinal mucosa or interfering with hosts’ appetite (Hall *et al*, 2008). In the same way, malnourishment itself may upsurge liability of contracting parasitic infections leading to this closed circle (Yones *et al*, 2015). Another interesting finding is the positive association between vomiting and diarrhea with multiple parasitic infections that may result in reduced nutrient existence in alimentary tract for a time enough for absorption (Stephenson *et al*, 2000).

Not only underweight children in developing countries carry the higher risk of parasitic infections and their morbidity but also in developed countries. A meta-analysis review revealed that > 5 years aged children showed more susceptibility to multiparasitism associated underweight (Dobner and Kaser, 2018). These findings contribute to the fact that under-nutrition and intestinal parasitism are fundamentally associated. While parasitic infections lead to impaired metabolism and absorption in the developing gastrointestinal mucosa of young children, defective nutrition and inadequate dietary intake will result in weight loss and debilitated immunity and increase susceptibility to infections.

The current study revealed many points of strength and limitation. A major strength is the choice of < 5-age group, as they are neglected in comparison to school children, which form the primary focus of plans and health action strategies nowadays in Egypt. An additional strength is the use of double concentration and permanent staining to increase the sensitivity of the microscopic examination, which is not usually performed in epidemiological studies.

On the other hand, the methodology was limited by missing some follow-up data, such as; monitoring changes in the weight, height over a period of time, pattern and quality of food consumption, which would determine better indicator of under nutrition and therefore better causality (Keiser *et al*, 2002). Other limitation was relying on single stool sample for diagnosis due to compliance reasons. Multiple samples per child were needed to overcome the underestimation of prevalence due to intermittent shedding of diagnostic stages (Steinmann *et al*, 2008).

Conclusion

The multi-parasitism is common among the Egyptian children less than five years with a significant positive correlation with underweight. The Egyptian Public Health Authorities must assess more details associated with the multiparasitism, such as infection intensity, diverse age groups and effects on the children responses, which open insights to enroll under five years children simultaneously with school children in the ongoing Egyptian Governmental Deworming Programs.

Contribution of authors: The authors contributed in all activities; paper idea, study design, collection of materials, methodology, writing and revising.

Conflict of Interest: The authors declared that they have no competing interests**.**

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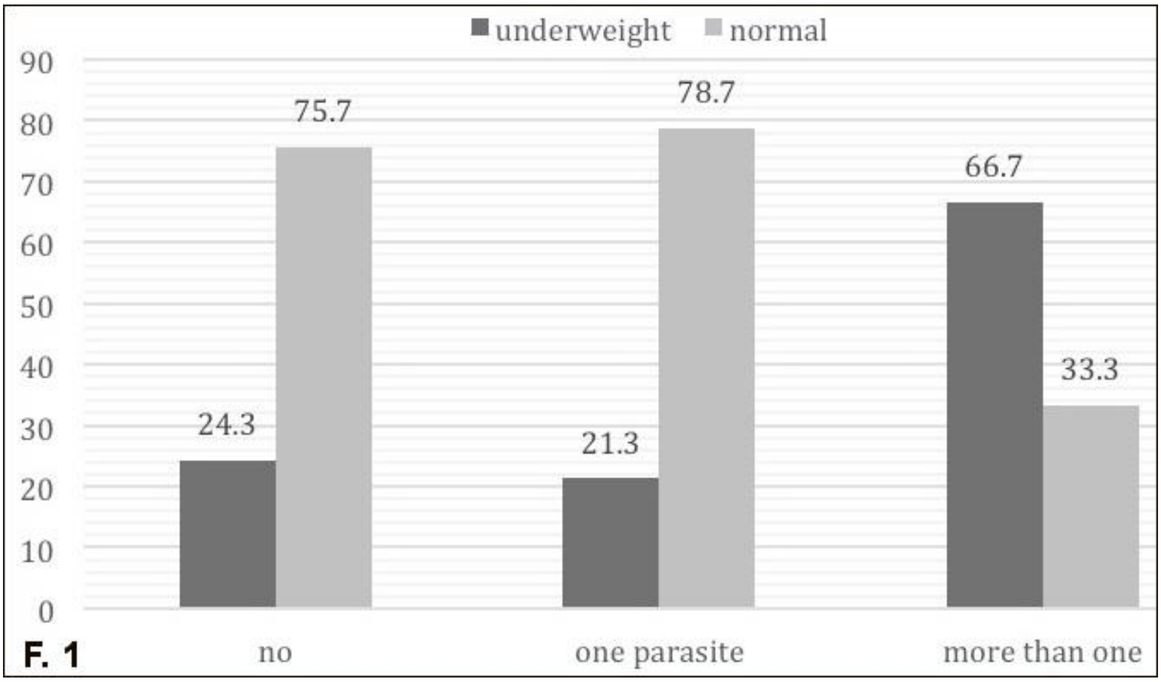
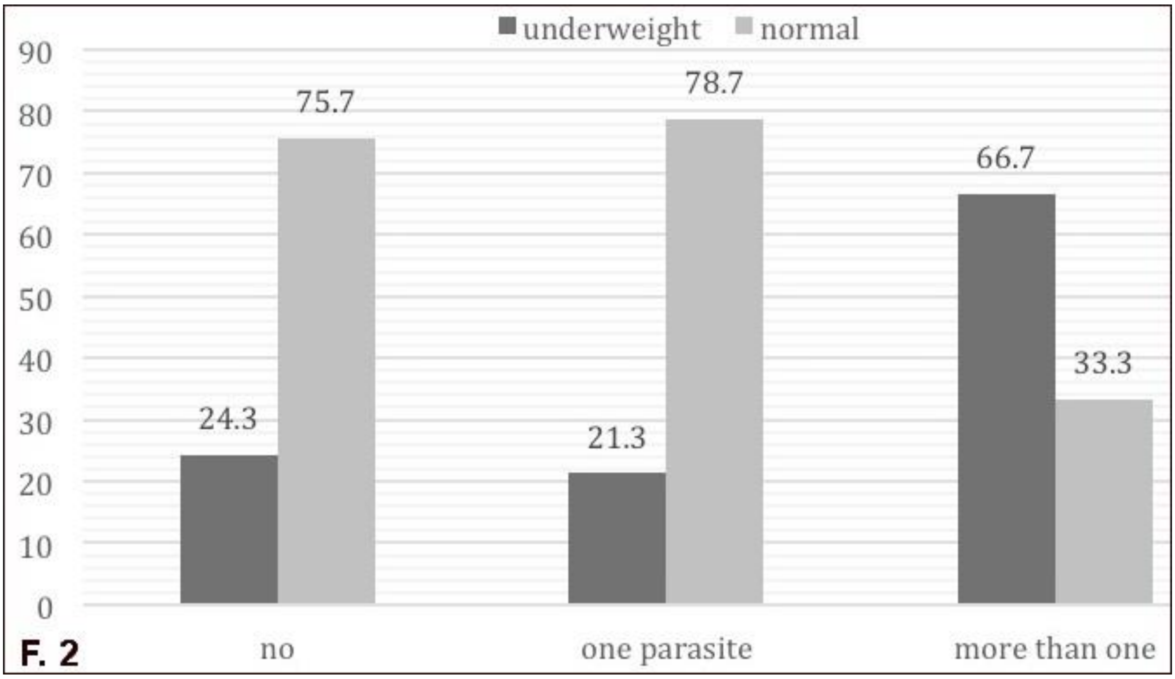
Explanation of figures

Fig. 1: Frequency of parasitic infections among stool samples
Fig. 2: Multiple parasitic infection profiles among normal and underweight children



F. 1



F. 2