

Microscopic Prevalence of Intestinal Parasites in Gastrointestinal Disordered Patients in Wasit Province, Iraq

Ghasik Aqeel *

Department of Microbiology, College of Medicine, University of Wasit, Wasit, Iraq.

*Corresponding Author

Ghasik Aqeel, Department of Microbiology, College of Medicine, University of Wasit, Wasit, Iraq.

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Abstract

Background: Intestinal parasites (IPs) have been a big concern for low-income countries as they are the major cause of high morbidity and mortality.

Aims: Identification the prevalence of IPs in gastrointestinal disordered patients, with estimation relationship of these pathogens to hematological and some epidemiological parameters.

Materials and Methods: A total of 200 individuals with a history of abdominal pain, anal itching, colic, diarrhea, dysentery, and nausea or vomiting were selected, and asked to obtain of fecal and blood samples. Traditional microscopic methods were used to detect the parasites; while, Automated Hematology Analyser was served for blood counting.

Results: There were 59.5% positive patients with intestinal parasites; in which, 68.91% with single infection (particularly *Entamoeba histolytica*, *Enterobius vermicularis* and *Giardia lamblia*), and 31.09% with mixed infections (particularly *Enterobius vermicularis* and *Ascaris lumbricoides* as well as *Giardia lamblia* and *Entamoeba histolytica*). Concerning risk factors, significant higher values of positivity were showed in patients of 3-7 years old, males more than females, and in rural more than urban areas. Regarding hematology, insignificant variation was recorded between infested and non-infested individuals, but macrocytic type of RBCs was highly prevalent in infested individuals. Subsequently, macrocytic type was increased significantly prevalent in patients with *Ascaris lumbricoides*; while, normocytic and microcytic types were seen in *Balantidium coli*. In patients with mixed infections, macrocytic type was recorded significantly in patients having *Enterobius vermicularis* and *Ascaris lumbricoides* as well as in those having *Giardia lamblia* and *Entamoeba histolytica*; while, microcytic type was identified in patients with *Enterobius vermicularis* and *Ascaris lumbricoides*, and those with *Enterobius vermicularis*, *Giardia lamblia* and *Balantidium coli*. However, normocytic type was seen significantly in patients with *Entamoeba histolytica* and *Balantidium coli*; *Entamoeba histolytica* and *Enterobius vermicularis*; and *Giardia lamblia* and *Entamoeba histolytica*.

Conclusions: The findings imply that intestinal parasites among gastrointestinal patients were prevalent as single or mixed infections. Age, sex and areas were related significantly with the existence of parasitic infection, in addition to the effect of these parasites on shape of RBCs. However, annual surveillance appears of great importance to detect the prevalence of intestinal parasites in different individuals.

Keywords: Neglected Tropical Diseases, Polyparasitism, Protozoa, Helminths, Macrocytic Anemia

Introduction

Intestinal parasites (IPs) are a group of pathogenic helminthic and protozoal species that inhabit the gastrointestinal region of human and other animals, which reproduce either sexually or asexually [1]. In general, these parasites either have single cell like unit that morphologically and functionally complete named protozoa; or consist of multicellular, bilaterally symmetrical, elongated flat or

round body known as helminths [2,3]. The parasitic infections can be existed throughout many countries to cause different infections that considered mostly as Neglected Tropical Diseases [4]. Worldwide, billions of people have been infected with intestinal parasites, particularly in poor areas due to fecal contamination of water, lack of adequate basic sanitation and environmental/ socio-cultural factors enhancing parasitic transmission [5,6]. Beside

of causing morbidity and mortality, transmission of intestinal parasitic infections have been accelerated by physical weakness and low educational performance of the poor segments of the populations; and intimately, linked with poverty, malnutrition, high population density, unavailability of potable water, poor environmental tropical climate and low altitude [7,8].

In acutely and chronically infections, individuals might asymptomatic or appeared with mild, moderate and sever signs; therefore, typical diagnosis relies on the microscopic detection of eggs, larval, trophozoite, cyst, and / or oocyst life stages in collected fecal samples on separate days remains the more common and available method as it simple, easy to perform and cheap assay [9,10]. In Wasit province (Iraq), several studies have been performed to detect of Ips [11-13], using of different diagnostic assays [14,15]; but with limited information. Hence, the current study was aimed to identify the prevalence of IPs in gastrointestinal disordered patients, with detecting the relationship of IPs to hematological and some epidemiological parameters.

Materials and Methods

Samples

Totally, 200 individuals of different ages (3-20 years) and both sexes (males and females) who having a history of digestive disorders (abdominal pain, anal itching, colic, diarrhea, dysentery, and nausea or vomiting) were subjected to the present study that conducted at Wasit province during January and April (2023). Fecal and blood samples that collected from each patient into plastic containers contained 10% formalin, and anticoagulant EDTA plastic tubes, respectively, were kept cooled until be tested as soon as possible. In addition, the main clinical and epidemiological data for each patient were documented.

Hematology

Automated Hematology Analyser (Sysmex, Germany) was served to indicate the effect of intestinal parasites on values of total Red Blood Cell count (RBC), Hematocrit (HCT), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

Microscopic Examination of Fecal Samples

1. *Direct saline/Iodine wet mount*: This method was made to detect the eggs and larvae of helminthes or the cysts and trophozoites of a parasite in prepared slides that examined at 10× of light microscopy [16].

2. *Acid fast stain*: This method was applied to identification of coccidian protozoa, in particular, the oocysts of *Cryptosporidium* species in prepared slides at 40× and 100× [17].

3. *Cellophane tape method*: The eggs or adults of pinworm parasite were observed by this technique, and the prepared slides were examined under light microscope 10× [18].

Statistical Analysis

The *t-test*, One-Way ANOVA and Odds ratio in the GraphPad Prism (version 6.0.1) Software were used to detect significant variation between values of study groups at $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***) and $P \leq 0.0001$ (****). Values were represented as either percentage (%) or Mean (M) ± Standard Error (SE), [19].

Results

Traditional diagnostic methods applied in this study for testing the fecal samples of 200 patients with digestive disorders, revealed that the total positive samples with intestinal parasites were 119 (59.5%) samples (Figure 1).

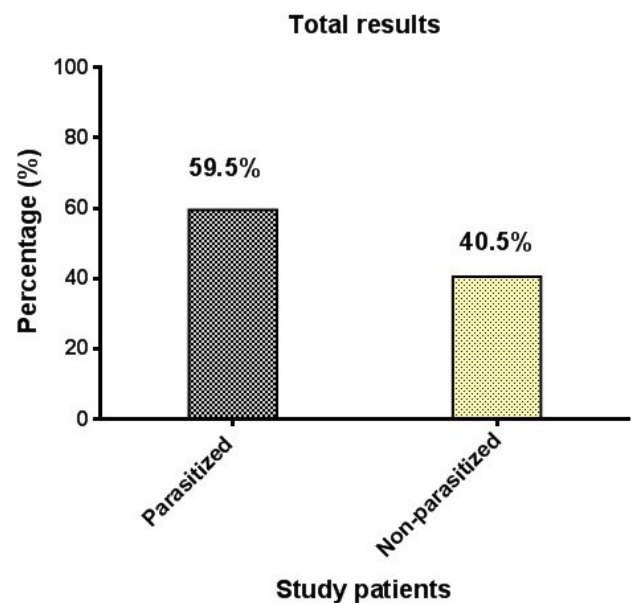


Figure 1: Total results for testing the fecal samples of study patients by the traditional diagnostic methods (Total No: 200).

Also, the findings showed that there was a significant increase ($P \leq 0.028$) in study parasitized patients infested with at least one type of intestinal parasites (single infection) 68.91% (82/119) when compared to those infested with more than one type of parasites (mixed infection), 31.09% (37/119), (Figure 2).

Among the parasitized patients with single infection, significant increases ($P < 0.05$) were reported in prevalence rate of *Entamoeba histolytica* (25.61%), *Enterobius vermicularis* (21.95%) and *Giardia lamblia* (20.73%); while significant reduction was seen in prevalence rate of *Ancylostoma duodenale* (4.88%), *Trichuris trichiura* (2.44%) and *Strongyloides stercoralis* (1.22%) in comparison to other intestinal parasites; *Ascaris lumbricoides* (10.98%), *Cryptosporidium* spp. (7.32%) and *Balantidium coli* (4.88%), (Table 1).

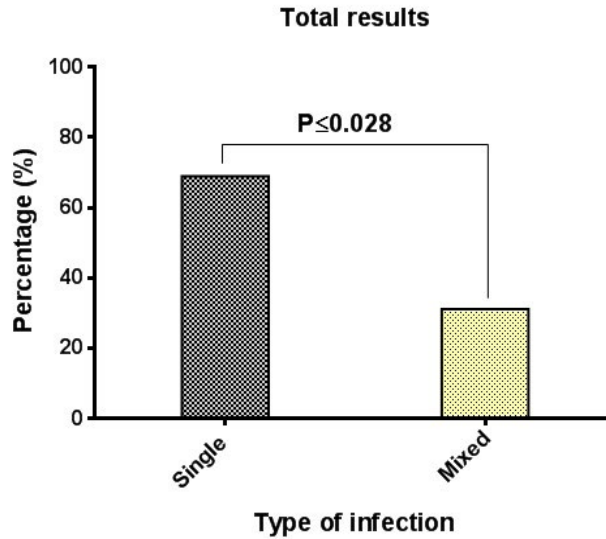


Figure 2: Total results for single and mixed infections among the parasitized positive patients (Total No: 119).

Parasite	Positive		P-value
	No.	%	
<i>Ancylostoma duodenale</i>	4	4.88	0.0112
<i>Ascaris lumbricoides</i>	9	10.98	
<i>Balantidium coli</i>	4	4.88	
<i>Cryptosporidium spp</i>	6	7.32	
<i>Entamoeba histolytica</i>	21	25.61 *	
<i>Enterobius vermicularis</i>	18	21.95 *	
<i>Giardia lamblia</i>	17	20.73 *	
<i>Strongyloides stercoralis</i>	1	1.22	
<i>Trichuris trichiura</i>	2	2.44	
Total No.	82	-	-

Table 1: Distribution of intestinal parasites among individuals infested with single infection (Total No: 82).

Concerning parasitized patients with mixed infection (polyparasitism), significant higher prevalence ($P < 0.05$) of parasites were detected in patients having *Enterobius vermicularis* and *Ascaris lumbricoides* (24.32%) as well as *Giardia lamblia* and *Entamoeba histolytica* (21.62%); while significant lowering was recorded in patients with *Ascaris lumbricoides* and *Balantidium coli* (0%), *Balantidium coli* and *Cryptosporidium spp.* (0%), *Cryptosporidium spp.* and *Entamoeba histolytica* (0%), *Ascaris lumbricoides* and *Entamoeba histolytica* (2.7%), *Balantidium coli*

and *Giardia lamblia* (2.7%), *Cryptosporidium spp.* and *Giardia lamblia* (2.7%), *Balantidium coli* and *Enterobius vermicularis* (5.41%), *Trichuris trichiura* and *Ascaris lumbricoides* (5.41%), as well as *Trichuris trichiura* and *Entamoeba histolytica* (5.41%). Other mixed infections were included *Entamoeba histolytica* and *Enterobius vermicularis* (13.51%); *Entamoeba histolytica* and *Balantidium coli* (8.11%); as well as *Enterobius vermicularis*, *Giardia lamblia* and *Balantidium coli* (8.11%), (Table 2).

Parasite	Positive		p-value
	No.	%	
<i>Ascaris lumbricoides</i> + <i>Balantidium coli</i>	0	0	0.0339
<i>Ascaris lumbricoides</i> + <i>Entamoeba histolytica</i>	1	2.7	
<i>Balantidium coli</i> + <i>Cryptosporidium spp.</i>	0	0	
<i>Balantidium coli</i> + <i>Enterobius vermicularis</i>	2	5.41	
<i>Balantidium coli</i> + <i>Giardia lamblia</i>	1	2.7	
<i>Cryptosporidium spp.</i> + <i>Entamoeba histolytica</i>	0	0	
<i>Cryptosporidium spp.</i> + <i>Giardia lamblia</i>	1	2.7	
<i>Entamoeba histolytica</i> + <i>Balantidium coli</i>	3	8.11	
<i>Entamoeba histolytica</i> + <i>Enterobius vermicularis</i>	5	13.51	
<i>Enterobius vermicularis</i> + <i>Ascaris lumbricoides</i>	9	24.32 *	
<i>Enterobius vermicularis</i> + <i>Giardia lamblia</i> + <i>Balantidium coli</i>	3	8.11	
<i>Giardia lamblia</i> + <i>Entamoeba histolytica</i>	8	21.62 *	
<i>Trichuris trichiura</i> + <i>Ascaris lumbricoides</i>	2	5.41	
<i>Trichuris trichiura</i> + <i>Entamoeba histolytica</i>	2	5.41	
Total No.	37	-	

Table 2: Distribution of intestinal parasites among individuals infested with mixed infection (Total No: 37).

Concerning age groups, significant higher values of positivity, Odds ratio and risk were showed in patients of 3-7 years old (73.97%, 2.711 and 1.445, respectively); while, lowered values were seen in patients of 13-17 (43.28%, 0.365 and 0.654, respectively) and 18-20 (41.67%, 0.463 and 0.688, respectively) years old (Table 3).

Group/year	Total No.	Positive		Odds Ratio	Relative Risk
		No.	%		
3-7	73	54	73.97 *	2.711 *	1.445 *
8-12	48	31	64.58	1.327	1.116
13-17	67	29	43.28	0.365	0.654
18-20	12	5	41.67	0.463	0.688
Total	200	119	-	-	-
p-value			0.0485	0.0001	0.0059

Table 3: Distribution of intestinal parasites among different age groups of study patients (Total No: 200).

Significantly, males were showed a higher positivity (65.19%), Odds Ratio (2.053) and Risk (3.216) to intestinal parasites than females (47.69%, 0.487 and 0.311, respectively), (Table 4).

Group/year	Total No.	Positive		Odds Ratio	Relative Risk
		No.	%		
Female	65	31	47.69	0.487	0.311
Male	135	88	65.19	2.053	3.216
Total	200	119	-	-	-
p-value			0.0411	0.0004	0.0001

Table 4: Distribution of intestinal parasites among both gender groups of study patients (Total No: 200).

Significant increasing in intestinal parasites was reported in patients of rural areas (69.23%) when compared to those of urban areas (57.14), (Table 5).

Group/year	Total No.	Positive		Odds Ratio	Relative Risk
		No.	%		
Rural	39	27	69.23	1.688	1.212
Urban	161	92	57.14	0.591	0.825
Total	200	119	-	-	-
p-value			0.0493	0.0002	0.0093

Table 5: Distribution of intestinal parasites among both regional groups of study patients (Total No: 200).

Regarding hematology, insignificant variation between infested and non-infested individuals was recorded in all parameters of blood (Table 6).

Parameter	Unit	Values (Mean ± Standard Error)		p-value
		Infested	Non-Infested	
RBCs	× 10 ⁶ /μl	3.91 ± 0.27	4.31 ± 0.85	0.0592
HCT	%	43.11 ± 1.32	40.28 ± 1.37	0.073
Hb	g/dl	11.15 ± 0.94	11.78 ± 0.96	0.0615
MCV	fl	110.27 ± 3.81	93.56 ± 3.45	0.0674
MCH	pg	28.52 ± 2.01	27.34 ± 2.11	0.0824
MCHC	g/dl	26.05 ± 2.68	30.01 ± 2.33	0.0585

Table 6: Values of RBCs parameters among infested and non-infested study patients (Total No: 200).

Based on morphology of RBCs, macrocytic type was prevalent in infested individuals (66.39%); whereas, normocytic type was seen in non-infested individuals (51.85%), (Table 7).

Group	Value [No. (%)]	
	Infested	Non-Infested
Macrocytic	79 (66.39%) *	7 (8.64%)
Normocytic	9 (7.56%)	42 (51.85%) *
Microcytic	31 (26.05%)	32 (39.51%)
Total	119	81
p-value	0.0271	0.0352

Table 7: Morphology of RBCs in infested and non-infested study patients.

In patients with single intestinal parasites, macrocytic type was more prevalent in patients with *Ascaris lumbricoides* (29.63%); while, normocytic and microcytic types were seen in *Balantidium coli* (66.67% and 31.82%, respectively), (Table 8).

Parasite	Type of RBC morphology		
	Macrocytic	Normocytic	Microcytic
<i>Entamoeba histolytica</i>	3 (5.56%)	1 (16.67%)	0 (0%)
<i>Enterobius vermicularis</i>	9 (16.67%)	0 (0%)	0 (0%)
<i>Giardia lamblia</i>	4 (7.41%)	0 (0%)	0 (0%)
<i>Strongyloides stercoralis</i>	3 (5.56%)	0 (0%)	3 (13.64%)
<i>Ascaris lumbricoides</i>	16 (29.63%)*	0 (0%)	5 (22.73%)
<i>Balantidium coli</i>	7 (12.96%)	4 (66.67%)*	7 (31.82%)*
<i>Trichuris trichiura</i>	12 (22.22%)	0 (0%)	5 (22.73%)
<i>Cryptosporidium spp.</i>	0 (0%)	1 (16.67%)	0 (0%)

<i>Ancylostoma duodenale</i>	0 (0%)	0 (0%)	2 (9.09%)
Total	54	6	22
p-value	0.0318	0.0274	0.0448

Table 8: Association of single infection with intestinal parasites to type of RBC morphology.

In patients with mixed infections, macrocytic type of RBCs was recorded significantly in patients with *Enterobius vermicularis* and *Ascaris lumbricoides* (28%) as well as in those with *Giardia lamblia* and *Entamoeba histolytica* (24%); while, microcytic type of RBCs was identified significantly in patients with *Enterobius vermicularis* and *Ascaris lumbricoides* (22.22%)

and those with *Enterobius vermicularis*, *Giardia lamblia* and *Balantidium coli* (22.22%). However, normocytic type of RBCs was seen significantly in patients with *Entamoeba histolytica* and *Balantidium coli* (33.33%); *Entamoeba histolytica* and *Enterobius vermicularis* (33.33%); and *Giardia lamblia* and *Entamoeba histolytica* (33.33%), (Table 9).

Parasite	Type of RBC morphology		
	Macrocytic	Normocytic	Microcytic
<i>Ascaris lumbricoides</i> + <i>Balantidium coli</i>	0 (0%)	0 (0%)	0 (0%)
<i>Ascaris lumbricoides</i> + <i>Entamoeba histolytica</i>	1 (4%)	0 (0%)	0 (0%)
<i>Balantidium coli</i> + <i>Cryptosporidium spp.</i>	0 (0%)	0 (0%)	0 (0%)
<i>Balantidium coli</i> + <i>Enterobius vermicularis</i>	1 (4%)	0 (0%)	1 (11.11%)
<i>Balantidium coli</i> + <i>Giardia lamblia</i>	1 (4%)	0 (0%)	0 (0%)
<i>Cryptosporidium spp.</i> + <i>Entamoeba histolytica</i>	0 (0%)	0 (0%)	0 (0%)
<i>Cryptosporidium spp.</i> + <i>Giardia lamblia</i>	0 (0%)	0 (0%)	1 (11.11%)
<i>Entamoeba histolytica</i> + <i>Balantidium coli</i>	2 (8%)	1 (33.33%)*	0 (0%)
<i>Entamoeba histolytica</i> + <i>Enterobius vermicularis</i>	3 (12%)	1 (33.33%)*	1 (11.11%)
<i>Enterobius vermicularis</i> + <i>Ascaris lumbricoides</i>	7 (28%)*	0 (0%)	2 (22.22%)*
<i>Enterobius vermicularis</i> + <i>Giardia lamblia</i> + <i>Balantidium coli</i>	1 (4%)	0 (0%)	2 (22.22%)*
<i>Giardia lamblia</i> + <i>Entamoeba histolytica</i>	6 (24%)*	1 (33.33%)*	1 (11.11%)
<i>Trichuris trichiura</i> + <i>Ascaris lumbricoides</i>	1 (4%)	0 (0%)	1 (11.11%)
<i>Trichuris trichiura</i> + <i>Entamoeba histolytica</i>	2 (8%)	0 (0%)	0 (0%)
Total	25 (65.57%)	3 (8.11%)	9 (24.32%)
P-value	0.0369	0.0282	0.0413

Table 8: Association of mixed infection with intestinal parasites to type of RBC morphology.

Discussion

Intestinal parasites are cosmopolitan in distribution, still establishes one of the important causes of public health problem in the world, especially in developing countries like Iraq. In this study, traditional diagnostic methods reported that the prevalence rate of intestinal parasites among the gastrointestinal disordered patients aged ≤ 20 years was 59.5%. Comparatively, the prevalence rate of IPs in Wasit province 47.7% by Rahi and Majeed [11], 47.17% by Jaffar et al. [12], 61.6% by Ahmed et al. [15]; whereas in other provinces, there were 55.6% in Sulaimani [20], 89.16% in Baghdad [21], 22.27% in Duhok [22], 18.93% in Karbala [23], and 36.83% in Al-Najaf [24].

This study focused on prevalence of single parasitic infections and mixed parasitic infections, as the positive our findings were 68.91% and 31.09%, respectively. However, single or mixed parasitic infections have impact on patient's health, anemia

condition, nutritional status cognitive development, work attendance and mortality. Also, application of advanced diagnostic assays such as molecular methods can detect more positive polyparasitism infections than microscopy [25]. For many years, the compartmentalization of scientists into specialists who work on one particular infection has formed a major roadblock to studying mixed infections [26]. However, the growing of awareness of the extent of mixed infections (polyparasitism), and in particular the difficulties it poses for the accurate interpretation of data generated in population studies that focus on single infections, has stimulated increasing numbers of researchers to face the challenge of studying polyparasitism [27].

Entamoeba histolytica, *Enterobius vermicularis* and *Giardia lamblia* were the most prevalent IPs in current study among the single infected patients; while, *Enterobius vermicularis* and *Ascaris lumbricoides* as well as *Giardia lamblia* and *Entamoeba*

histolytica were the more common parasites in polyparasitism patients. Several studies demonstrated the higher prevalence of these species in gastrointestinal patients, indicating that different regions in Iraq are endemic to these species.

Our results showed that male children (3-7 years old) of rural areas are more positivity than other opposite groups of risk factors; which similarly to those observed by other researchers [12,15,21], but in contrast to those reported by others [11,24,28]. This variation might be attributed to method and criteria of sampling, differences in environmental and socio-ecological conditions, as well as health status and level of immunity of participants. Subsequently, the presence of other metabolic or infectious diseases might significantly increase the severity of parasitic infections.

Although, insignificant variation was showed in hematological parameters of infested and non-infested individuals of the present study, macrocytic anemia was showed in infested individuals and related particularly to some species of IPs. These findings that in agreement with a number of researchers [29] and contrast to others [28,30], might be attributed to the low burden of IPs, species of IP, chronic nature of an infection, and high level of immunity. Also, the high prevalence of IPs among study population might be independently associated with anemia and malnutrition. Several studies referred to the role of some species of IPs in nutrient and mineral deficiency such as iron, folate, vitamin-B₁₂, zinc and proteins [31-35].

Conclusion

The findings imply that intestinal parasites among gastrointestinal patients were prevalent as single or mixed infections. Age, sex and areas were related significantly with the existence of parasitic infection, in addition to the effect of these parasites on shape of RBCs. However, annual surveillance appears of great importance to detect the prevalence of intestinal parasites in different individuals.

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