

Intestinal Protozoa (*Entamoeba histolytica* and *Giardia lamblia*) and their effect on some Hematological Parameters in Al-Manssora Hospitals, Aden, Yemen

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DOI: <https://doi.org/10.47372/uajnas.2022.n2.a08>

Abstract

Intestinal protozoa are among the organisms that cause diseases in various countries of the world, especially poor countries. Six hundred and three stool samples were collected from patients coming to some hospitals in Mansoura District (22 May Hospital, Cuban Hospital, Al-Naqeeb Hospital, Al Salam Hospital), and blood samples were taken from 384 patients of both sexes infected with intestinal protozoa (*E. histolytica*, *Giardia lamblia*) and 21 blood samples from non-infected people.

The results of the current study showed that the total infection rate of intestinal protozoa (*E. histolytica*, *G. lamblia*) was 603/384 (63.68%). For blood picture tests, a decrease in haemoglobine values and an increase in the total number of white blood cells and Eosinophils were observed for the affected people. The aim of this study is to determine the prevalence rate of *E. histolytica* and *G. lamblia* and its effect on some hematological parameters in some hospitals in Al-Mansoura District of Aden Governorate (Yemen).

We recommend raising the level of health by providing educational programs and paying attention to personal hygiene in order to reduce the incidence of parasites, activate the role of health institutions, and work to combat rodents and insects to cut off the parasite life cycle.

Keywords: *Entamoeba histolytica*, *Giardia lamblia*, Hemoglobin, White blood cells, Eosinophils.

Introduction

Infection with intestinal protozoa has worldwide distribution and is particularly common in tropic and sub-tropic areas of the world, with millions of cases of diarrhea occurring in each year and in people with low social, and economic conditions and poor living conditions, as well as people in crowded areas with poor sanitation, improper disposal of garbage, unsanitary water supplies and unhealthy personal habits (11; 21)

Protozoan infections were amongst the leading causes of morbidity and mortality throughout the world (19). With more than 58 million diarrheal cases detected each year (23). However, it was difficult to estimate the actual burden of protozoan infections due to under reporting. Moreover, intestinal protozoan infections contribute to malnutrition, protein and iron deficiencies, an increment on health costs, as well as long-term deleterious effects (9).

The amoebic infection and giardiasis were one of the intestinal protozoa that cause public health problems in most developing countries as well as some developed countries *G. lamblia* and *E. histolytica* is considered to be one of the leading causative agents of diarrhoea in both children and adults (6; 7).

Infection with intestinal parasites has a significant effect on the values of white blood cells (WBC) components, eosinophils and hemoglobin (Hb) (5). Hemoglobin loss occurs in some pathological conditions including: parasitic infection, malnutrition, blood loss (3). Other components, such as packed cell volume (PCV), has relation with anemia where the loss of PCV occurs as a result of specific conditions such as anemia (14). White blood cells are considered as one of the basic components of blood and are presented in peripheral blood (12). to provide

defense against parasites and other diseases (15). White blood cells vary in size, proportions and functions, which are Neutrophils, Eosinophils, Basophils, Lymphocytes and Monocytes (14).

Materials and Methods

Study area:

Aden governorate is located along the southern coast of the Republic of Yemen. It lies at (12⁰ - 47'N) latitude and (44⁰ - 58'E) longitude and is a semi island (20). Aden is about 363 kilometers far from the capital Sana'a. It occupied about 750 km² and is divided into eight districts. The population of Aden governorate is 684,322 (27).



Figure (1). Map of Aden governorate adapted by www.europa.uk.com, showing the location of the directorate of Al-Mansoura

Questionnaire

The subjects from whom stool and blood samples were taken were questioned by preparing a questioners asking patients to answer questions related to: name, gender, and ages. residence.

Stool Collection

Clean plastic cups were used for stool samples collection (5-10g) avoiding presence of urine or any other substances that may lead to false results.

Stool Examinations

Macroscopicaly Examination

It was performed by observing grossly the consistency of stool samples, presence of protozoa, blood, mucus.

Microscopicaly Examination

Wet preparation technique (unstained preparation)

About 2 mg of faces were mixed with one or two drops of physiological saline (0.9 gm/dl) on a slide and covered with a cover-glass and examined microscopicaly, using low power objective lens (10X), followed by high objective lenses (40X), at least three smears were examined for each sample (13).

Formalin – Ether sedimentation technique:

All specimens were kept in a refrigerator and set at 4 °C for not more than 24 hour before examination. These specimens were used for formalin-ether sedimentation. Approximately 2 g of

stool were emulsified in a wax-paper cup, using applicator sticks in 20 ml of distilled water. The methods were done as described by (24).

Each specimen was examined microscopically by staining preparation with, 2% iodine solution, methylene blue 0.06%, eosin stain 5g/l(0.5%w/v), and malachite green, 5g/l (0.5% w/v) (8; 10).

Blood Examinations

Blood samples collection:

Blood samples were collected from the 384 patients with intestinal protozoa, a sample of blood consisting of 2 milliliters was obtained from vein by a sterile disposable syringe from each patient. The blood sample was poured into EDTA. anticoagulated tubes for WBC count, Eosinophil count and Hb determination (22).

Blood picture examinations:

These examinations were performed for a certain number of infected patients with intestinal protozoa disorders. About 2 ml of the whole blood was collected into EDTA anticoagulated tubes to perform the (CBC).

Statistical analysis

Statistical analysis of the data was performed using t-test, ANOVA and LSD were applied to find the significant difference between the data by using the statistical program SPSS (version 21).

Results

The prevalence rates of intestinal protozoa infections was (63.68%) .This study records that the number and percentage of parasite were follows: *Entamoeba histolytica* 162(26.87%), *Giardia lamblia* 60(9.95%), CO-infection 120 (19.90%), Multi infection 42 (6.96%) (Table 1). The results shows the percentage of intestinal protozoan infection in both males and females, as the infection in males was higher than that of females, with a males rate reaching 201/603 (33.34%) and a females rate of 183/603 (30.34%), and a statistical differences that did not reach a significant degree. ($t = 1.042$, $P = 0.298$) and in both sexes.

The infection with *E. histolytica* was the highest, with a males rate 84/603 (13.93%) and 78/603 (12.94%) a females, followed by co-infection (*E. histolytica* + *G. lamblia*,) which was a males rate 54/603 (8.96%) and 66/603 (10.94%) in females.

Followed by *G. lamblia*, with a males rate 39/603(6.47), and 21/603 (3.48%), in females. While the rate of multi infection was *E. histolytica* +*G. lamblia* +*E. Coli* with a males rate 9/603 (1.49%) and 7/603 (1.16%) in females, *E. histolytica* +*G. lamblia* + *E. vermicularis* with a males rate 6/603 (1.00%) and 4/603 (0.66%) in females, *E. histolytica* +*G. lamblia* + *Strongyloides* with a males rate 4/603 (0.66%) and 2/603 (0.33%) in females, *E. histolytica* +*G. lamblia* +*A. lumbricoides* with a males rate 3/603(0.50%) and 2/603 (0.33%) in females, *E. histolytica* +*G. lamblia* +*H. nana* with a males rate 2/603(0.33%) and 3/603 (0.50%) in females. It was also found in both sexes, the other parasites, where its percentage was 129 /603 (21.39%) with a males rate and 90 /603 (14.93%) in females.

Statistically and by using the chi-square test (χ^2), the results showed significant differences in parasite infection *Giardia lamblia* .($\chi^2= 5.400$, $P= 0.020$).

Table (1) Prevalence of intestinal protozoan infections and the other parasites by Sex in some Al-Manssora hospitals Aden, Yemen

Parasites	Gender			X ²	P-value
	Male (n 330) NO (%)	Female (n273) NO (%)	Total (n=603) NO (%)		
Single Infection					
<i>Entamoeba histolytica</i>	84 (13.93)	(12.94)78	(26.87)162	0.222	0.637
<i>Giardia lamblia</i>	(6.47)39	(3.48)21	(9.95)60	5.400	0.020
CO- infection					
<i>E. histolytica</i> + <i>G. lamblia</i>	(8.96)54	(10.94)66	(19.90)120	1.200	0.273
Multi infection					
<i>E. histolytica</i> + <i>G. lamblia</i> + <i>Entamoeba coli</i>	(1.49)9	(1.16)7	(2.65)16	0.250	0.617
<i>E. histolytica</i> + <i>G. lamblia</i> + <i>Strongyloides</i>	(0.66)4	(0.33)2	(0.99) 6	0.667	0.414
<i>E. histolytica</i> + <i>G. lamblia</i> + <i>A. lumbricoides</i>	(0.50)3	(0.33)2	(0.83) 5	0.200	0.655
<i>E. histolytica</i> + <i>G. lamblia</i> + <i>H .nana</i>	(0.33)2	(0.50)3	(0.83) 5	0.200	0.655
<i>E. histolytica</i> + <i>G. lamblia</i> + <i>E. vermicularis</i>	(1.00)6	(0.66)4	(1.66)10	0.400	0.527
Total	201 (33.34 %)	183(30.34%)	(63.68) 384	-	

NO = Number

Significant at P-value ≤ 0.05

X² = Chi-Square

Effect of intestinal protozoa infection (*E. histolytica* and *G. lamblia*) on blood picture

The patients infected with intestinal protozoa were divided according to age groups into three groups that included 66 (1-9) years, 99 (10-19) years, 219 (20 -67) years, and using ANOVA test. It was found that there are significant differences in the values of HB, WBC. Eosinophil when comparing different age groups at the level of significance ($P=0.025$), ($P=0.015$), ($P=0.000$), (**Table 2**).

The results showed that there was a decrease in the HB rate for the age group (1-9) year compared to the rest of the other age groups, as it reached (10.97 g/ dL), as reached its percentage in the age group (20-67) years (11.49 g / dL), then the age group (10-19) years (13.58 g / dL) (**Figure 2**).

A high rate of WBC was observed in the age group (1-9) years compared to the rest of the age groups, reaching an average of ($8.22 \times 10^3 / \mu\text{L}$), followed by the age group (10-19) years. ($7.71 \times 10^3 / \mu\text{L}$), and the lowest average for the age group was (20-67) years ($7.09 \times 10^3 / \mu\text{L}$) (**Figure 3**).

As for Eosinophil, the highest rate for the age group (10-19) years was 5.74, followed by the age group (20-67) in 5.68 rate, and the lowest rate was for the age group (1-9) years, with a rate of 4.65 (**Figure 4**).

Table (2): The blood picture analysis for intestinal protozoa infection (*Entamoeba histolytica* and *Giardia lamblia*)
 LSD = P < 0.05 (P = 0.000, P = 0.000, P = 0.032, P = 0.011, P = 0.020, P = 0.007)

Subject	NO (384)	Age rang	T.WBC		P. value	Eosinophil		P. value	HB		P. value
			Mean ±S.D	Rang		Mean ±S.D	Rang		Mean ±S.D	Rang	
Group (1)	66	1-9	8.22± 3.69	(3.75-16.70) 12.95	0.025	4.65±1.79	(1.1-8.0) 6.9	0.015	±1.9410.97	(6.5-15.5) 9.0	0.000
Group (2)	99	10-19	7.71± 2.95	(3.56-16.17) 12.61		±2.835.74	(1.3-15.0) 13.7		±1.5113.58	(10.2-17.3) 7.1	
Group (3)	219	20-67	±3.0087.09	(3.42-17.30) 13.88		±2.815.68	(0.1-13.2) 13.1		±1.7711.49	(7.7-16.1) 8.4	

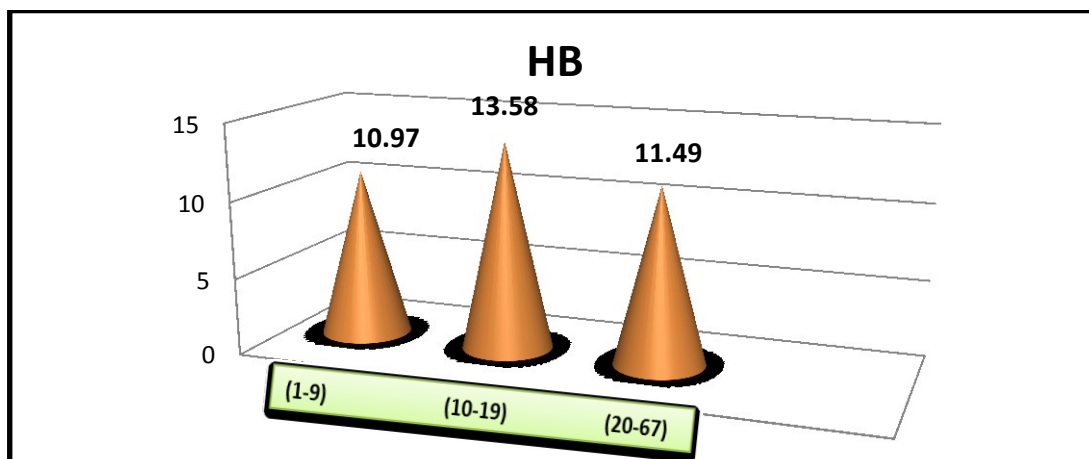


Figure (2): HB, WBC, and Eosinophils level for age groups

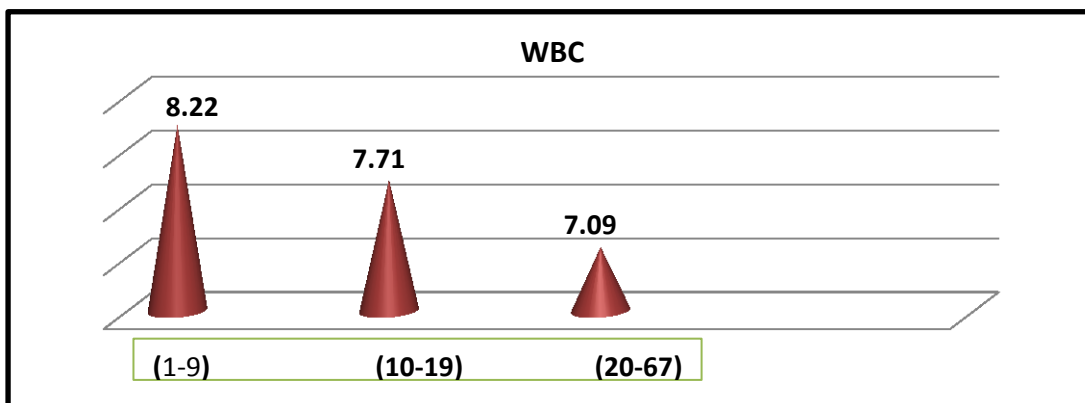


Figure (3): WBC level for age groups

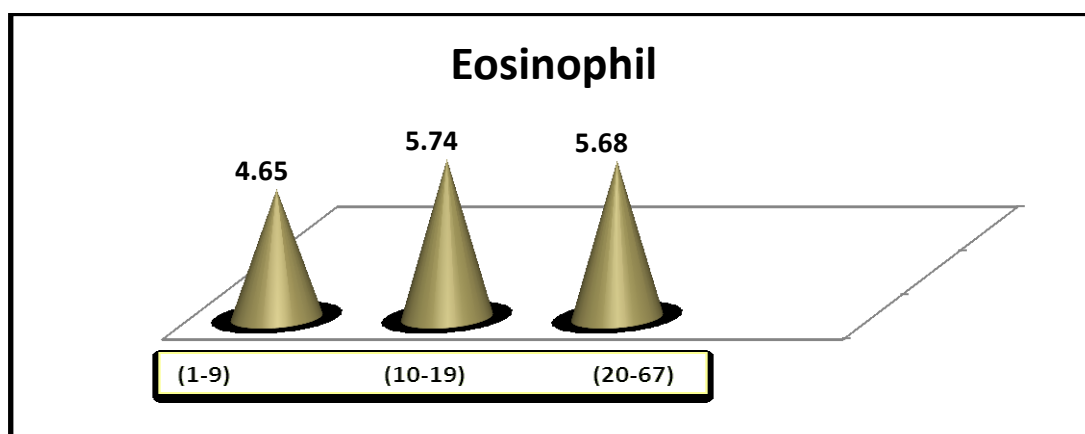


Figure (4): Eosinophils level for age groups.

I find out the direction of the differences between age groups, a statistical analysis was used to test the least significant difference (LSD) at the level of significance ($P < 0.05$) with respect to hemoglobin (HB).

Significant differences were found between the age groups (1-9) years and (10-19) years ($P = 0.000$), between the age groups (20 -67) years, and (10-19) years ($P = 0.000$), and between the age group (1-9) years and (20 -67) years ($P = 0.032$).

For WBC, significant differences were found between the age groups (1-9) years, (20-67) years, and (10-19) years ($P = 0.011$). As for Eosinophil, significant differences were found between the age groups (10-19) years (1-9) years ($P = 0.020$), and between the age groups (20 -67) years (1-9) years ($P = 0.007$).

Table (3) shows the difference in the HB rate for those infected with intestinal protozoa and non-infected and by using the *t. test*, it was found that there was a significant difference at the level of significance ($P = 0.000$). Also, it was observed that there is a decrease in the HB rate of the infected compared to the uninfected, as the HB rate reached (11.94g / dL) in the infected and (13.57g / dL) the non-infected.

Table (3) also shows the difference in the WBC rate for those infected with intestinal protozoa and non-infected, and using the *t. test*, it was found that there was a significant difference at the level of significance ($P = 0.000$). It is also observed that there was increase in the WBC rate of the infected, compared to the uninfected, as the WBC rate was reached in the infected ($7.44 \times 10^3 / \mu\text{L}$) and the non-infected ($6.17 \times 10^3 / \mu\text{L}$).

Table (3)also shows the difference in the Eosinophils rate for those infected with intestinal protozoa and non-infected, and using the *t. test*, it was found that there was a significant difference at the level of significance ($P = 0.004$). Also observed that there was an increase in the Eosinophils rate of the infected compared to the uninfected, as the Eosinophils rate was reached in the infected (398.16 cell/ mm^3) and the non-infected (271.623 cell/ mm^3).

Table (3): Level of HB, WBC and Eosinophils for infected patients with intestinal protozoa infected and non-infected

HB ± S.D Mean	Non infected No.	HB ± S.D Mean	Infected No.	P. value
13.57±1.81	21	11.94±1.99	384	0.000
W.B.C ± S.D Mean		W.B.C ± S.D Mean		
6.17±0.97	21	7.44±3.14	384	0.000
Eosinophils Per /mm ² values ± S.D mean		Eosinophils Mean± S.D		
271.623±138.28	21	398.16±233.22	384	0.004

Discussion

The results of the current study showed significant changes in haematological parameters patients with intestinal protozoan infection (*E. histolytica* and *G. lamblia*). In comparison with the results of the control group, it was observed a decrease in the Hb level and an increase in the number of (WBC), and eosinophil in the affected people, where it reached (11.94g / dL), ($7.44 \times 10^3 / \mu\text{L}$) and (398.16 cell/ mm^3). This result was similar to those of (26, 29 & 16), how studied the effect of intestinal parasites on the blood picture as our study agrees with (17). A study in Sudan conducted to find out the effect of *E. histolytica* on some haematological parameters, showed a

decrease in the rate of Hb and an increase in WBC and eosinophil in people with *E. histolytica* parasite.

A study of (28) from Syria was conducted to find out the prevalence of anaemia in giardiasis intestinalis in Syrians children. The results show a decrease in the rate of Hb to (11.3g /dl) in children with intestinal giardia, compared to the healthy group (13.5g /dl). A study of (30) in Iraq, found that infection with the *E. histolytica* parasite resulted in a decrease in the amount of Hb for some age groups infected to (9.90g/dl) and a rise in WBC to (699.920 cell/ mm³), Likewise, the height of Eosinophil, compared to the healthy group. A study of (2) in India was conducted to find out impact of giardiasis on hematological profile of infected children. The results of his study showed a significant decrease in mean values of Hb from (11.15) to (10.05) in males and (11.36) to (10.08) in females. In case of WBC count, a significant increase in the total number of WBC was observed in a case of infected children from (7.96) to (9.38), with respect to differential leukocyte count, Eosinophils showed a marked increase in their number in infected individuals (5.0), in comparison to uninfected ones (2). Another study (31) in Iraq was conducted to know the relationship of intestinal protozoan infection with blood parameters to detect bowel performance, and the results showed a decrease in the rate of Hb and an increase in the number of WBC and Eosinophilia in patients affected with *G. lamblia* and *E. histolytica*. A study conducted in Iraq (1, 32, 25) to know the hematological variables in infected patients with *E. histolytica* parasite. The results of the studies showed a decrease in the rate of Hb in those with infected.

The results of our study also showed that the age group (1-9) years was the most age group in which Hb decreased as well as the most age group in which WBC increased. Our current study is in agreement with what stated above with (26), where the small age groups (ages 6-7) were the lowest in the Hb level as they reached (9.6g /dL), while the age group (4-5) years was the most an increase in the number of WBC, amounted to (9600 cell / mm³). Abd Sadah (1) reported that, the age group (under 10 years old) is the most age groups the Hb rate has decreased to (9.6g /dl), and our study differs with other studies such as (4). where the results of the study showed that the large age groups (61-70) years were the ones whose the Hb rate decreased (9.90 g / dl) and the rise in WBC and eosinophil for the age group (51-60) years and the study of (31) showed the age group (more than twenty years >20) was the most affected by most blood parameters.

Conclusion

The current study showed the prevalence of intestinal protozoa infection in some hospitals in Mansoura district, Aden governorate, and the prevalence rates of intestinal protozoa infection were (63.68%) more common than *Entamoeba histolytica*, followed by *Giardia lamblia*. The results of the current study showed significant changes in the results of hematological criteria with people intestinal protozoan infection (*E. histolytica* and *G. lamblia*). In comparison with the results of the healthy group, where it is observed a decrease in the Hb level and an increase in the number of (WBC), and Eosinophil in the affected people, reached (11.94g / dL), ($7.44 \times 10^3 / \mu\text{L}$) and (398.16 cell/mm³).

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الأوليات المعوية (*Entamoeba histolytica* and *Giardia lamblia*) وتأثيرها

على بعض العوامل الدموية في مستشفيات المنصورة، عدن، اليمن

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DOI: <https://doi.org/10.47372/uajnas.2022.n2.a08>

المخلص

تعد الطفيليات المعوية من بين الكائنات الحية المسببة للأمراض في مختلف دول العالم، وخاصة البلدان الفقيرة. تم جمع 603 عينات براز من المرضى الوافدين إلى بعض مستشفيات مديرية المنصورة (مستشفى 22 مايو، المستشفى الكوبي، مستشفى النقيب، مستشفى السلام) وتم أخذ عينات دم من 384 مريضاً من كلا الجنسين من المرضى المصابين بالأوليات المعوية (اميبيا الزحار، الجيارديا لامبليا)، و 21 عينة دم من أشخاص غير مصابين.

أظهرت نتائج الدراسة الحالية أن نسبة الإصابة الكلية بالأوليات المعوية (*Entamoeba histolytica* و *Giardia lamblia*) كانت 384/603 (63.68%). بالنسبة لفحوصات الصورة الدموية لوحظ انخفاض في قيم الهيموجلوبين وارتفاع في العدد الكلي لخلايا الدم البيضاء والخلايا الحامضية للأشخاص المصابين.

الهدف من هذه الدراسة هو دراسة معدل انتشار (*Entamoeba histolytica* and *Giardia lamblia*) وتأثيرها على بعض العوامل الدموية في بعض مستشفيات مديرية المنصورة بمحافظة عدن (اليمن). نوصي برفع المستوى الصحي من خلال توفير برامج توعوية والاهتمام بالنظافة الشخصية لتقليل الإصابة بالطفيليات، وتفعيل دور المؤسسات الصحية، والعمل على مكافحة القوارض والحشرات لقطع دورة حياة الطفيل.

الكلمات المفتاحية: *Entamoeba histolytica*، *Giardia lamblia*، الهيموجلوبين، خلايا الدم البيضاء، الخلايا الحامضية.