Study the infection with intestinal parasites (*Entamoeba histolytica*) and its effect on the blood nature in children from Thi-Qar province

Yahya T. Daoud Khalid M. Dakhil Hanaa Daaj Khalaf Al-Mozan
(Received 21/2/2012, Accepted 18/3/2012)

الخلاصة

تضمنت الدراسة الحالية جمع 1001عينة براز لأطفال بعمر السبع سنوات فما دون . جمعت 333 عينة دم للذين ثبتت إصابتهم بالطفيليات المعوية من عينات الدراسة و100عينة دم من غير المصابين المراجعين لمستشفيات مناطق مختلفة في محافظة ذي قار، للمدة المحصورة بين الأول من تشرين الأول 2010 إلى نهاية تموز 2011 لدراسة نسب الإصابة بالطفيليات المعوية وتأثيرها في بعض معايير الدم . فحصت عينات البراز بطريقتين هما المسحة المباشرة باستخدام المحلول الملحي Normal Saline وصبغة اليود Lugol's Iodine وغير المباشرة مثل التطويف باستخدام كبريتات الخارصين المائية $ZnSO_4.7H_2O$ وأظهرت النتائج إن النسبة المئوية للمصابين بالطفيليات المعوية كانت (33.3%) شكلت الـ *Entamoeba histolytica* أعلى *نسبة* (30%) . اتضحت الفروق المعنوية باستخدام التحليل الإحصائي (T- test) بمستوى دلالة $P<0.05$ عند دراسة تأثير نوع الإصابة ونوع الطفيلي في بعض المعايير الدموية مثل الهيموكلوبين Hb وحجم الدم المضغوط PCV والعدد الكلي لكريات الدم البيض WBC وأعداد الحمضات. ولكون *E.histolytica* الطفيلي الأكثر شيوعا في هذه الدراسة ارتأينا تسليط الضوء على تأثير ذلك الطفيلي في كريات الدم الحمر وتركيز الأجسام المضادة التي يكونها الجسم ضده ، إلا ان التحليل الإحصائي (T-test) لم يجد أي فروق معنوية في معايير كريات الدم الحمر للمصابين وغير المصابين بذلك الطفيلي وكذلك الحال لتركيز IgG بينما وجد ذلك الفرق في تركيز IgM للمصابين مقارنة بغير المصابين .

Abstract

The present study has included collection of 1001 fecal samples of children aged seven years and less than that age . 333 blood samples have been collected for the children that proved their infection by intestinal parasites from samples of study and 100 blood sample from non - infected who visited different hospitals in Thi – Qar Provence during the period from the first of October 2010 to the end of July 2011 to study the rate of intestinal parasites infection and their impact on certain criteria bloody . The fecal samples have been examined by two methods are: direct smear using Normal Saline and Lugol's Iodine and indirect with flotation by using Zinc Sulfate water $ZnSO_4. 7H_2O$. The results have showed that percentage of infected children with parasites was % 33.3 . *Entamoeba histolytica* formed high percentage % 30 . Significant differences by using (T-test) in level $P< 0.05$ become clear when studying effect type of infection and type of parasite on some criteria bloody such as Packed Cell Volume PCV , Hemoglobin Hb, White Blood Cell WBC and Number of eosinophile. *E. histolytica* formed high percentage therefore we decided to highlight on the impact of that parasite in red blood cells and the concentration of antibodies formed against it , but the statistical analysis did not find any significant differences in the criteria for red blood cells between infected and not infected with this parasite, as well as the concentration of IgG while we found IgM concentration difference in patients compared to non-infected.

Introduction

Intestinal parasitic infection is a serious public health problem throughout the world particular developing countries. Intestinal parasites are frequently transmitted via contaminated food and drinking water, but may also be spread from person to person through fecal-oral contact [1] . These parasites cause several symptoms, including : - diarrhea, fever, vomiting and anemia [2] . *Entamoeba*

histolytica is the most prevalent from all parasites in the world, particularly in the tropics and subtropics, causing Amoebiasis, Amoebic dysentery, Amoebic liver abscess and the disease comes after Malaria and Bilharziasis disease Schistosomiasis in causing death cases per year [3, 4] . Amoebiasis is a disease affects about 500 million cases a year, leading to the death of 100,000 nearly per year . Protozoa infection are concentrated in the younger groups due to a weakened immune system and lack of knowledge of health issues [5,6] . Anemia in children can be caused by iron deficiency and by health factors such as parasite infection [7] . Many studies have shown that hook worm causes chronic intestinal blood loss [8] , blood loss can also occur in *Trichuris* infection [9]. Parasitic infection stimulates a strong immune response against the parasite *E.histolytica* and that response is presence of antibodies, such as a class Immune globulin A [10] .

Material and Methods

Stool samples: Specimens should be collected in suitable clean containers, avoiding contamination with urine, water or disinfectants .

1 – Macroscopic examination

Feces should be examined for its consistency , color, odor, and presence of blood or mucous . In some instance, parasites may be seen on gross inspection as in the case of round worm, pin worm or tape worm proglottides [11, 12] .

2 - Microscopic examination

A - Direct method by using the normal saline and the logal, s iodine [13, 14].

B – Indirect method by using zinc sulfate centrifugal floatation [11,12].

Blood samples:We drawl of 2 ml of blood by intravenous injection of sterile medical and the transfer of about 0.5 ml plastic tubes to a container on the anticoagulant EDTA (Ethylene Diamine Tetra Acetic Acid) and put the rest in plastic tubes free of anticoagulant for the purpose of isolating the serum was performed the following tests: -

1- Examination of blood

A - Packed red blood cell volume or Microhematocrit Determination [15,16] .

B - Measurement of Hb

Hb was measured by a Reflotron plus the factory by the German company Roche established in accordance with the instructions accompanying the company.

C – Total Leukocytes count [17] .

D - Differential count of white blood cells [16,18,19] .

E - Measuring some of the criteria for red blood cells infected with *E. histolytica*

It measured by changes in red blood cells (Hb, MCV, MCH, MCHC, RDW) using a Coulter factory by the Japanese company Nihon Kohder established in accordance with the instructions that came with it.

2- Radial immune diffusion test by method Endoplast

We have identified some of immune globulin concentration IgM 35 sample and IgG 20 sample in the patients with sero-parasite *Entamoeba histolytica* using a kit consisting of all immune globulin .

Results

Statistical analysis (T-test) level of significance $P < 0.05$ revealed to the existence of significant differences for the effect of type of infection and the type of parasite on the components of the blood in Hb at infection with *T. hominis* reached %13.4 and the lowest value was %8.3 for *E. histolytica* + *H.nana* and the highest WBC was 13000 by infection *Entamoeba histolytica* + *Giardia lamblia* as for the lowest WBC recorded 4500 in *E.histolytica* + *G. lamblia* + *T.hominis* as for Number of eosinophile represented 540 the highest number for it with *E.*

histolytica + *H.nana* and recorded 0 in case of infection with *E.histolytica* + *G.* lamblia + *T.hominis* Table (1) .

No significant differences by using statistical analysis (T-test) between infected and not infected by the criteria of red blood cells where The T tabulated is equal to 1.78 higher T calculated was equal to 1.65 Table (2).

The emergence of RBCs, Hb, MCH, MCHC, Hct, MCV, RDW of (male and female) with (4.96, 4.87), (12.12, 11.37), (27.35, 25.6) (35.97, 35.37), (37.37, 35.12), (75.97, 72.32), (13.22, 14.05), respectively. Depending on the extraction of tabular t by 1.89 and the fact that the top of T calculated equal to 0.99 therefore the statistical analysis denies the existence of moral differences Table (3).

The back of the rate of concentration of IgG in the sero-infected children with *E.histolytica* of 1024.19 mg / Dessie L and the extent of 2115.50 mg / Dessie L and the average concentration samples control 990.29 extent of 1014.30 mg / Dessie liters. It shows the statistical analysis (T-test) level of significance $P<0.05$ there is no significant difference in IgG concentration between infected and control samples Table (4).

The average concentration of IgM in the sero-infected children with E.histolytica 116.78 mg / Dessie liters extent of 207.30 mg / Dessie L In the non-infected (control) was the average concentration 951.38 mg / Dessie L and the extent of 1014.30 mg / Dessie liters. According to that statistical analysis proved the existence of significant difference for the IgM concentration Between infected and not infected Table (5) .

Table (1) The impact of type of injury and the type of parasite in some components of the peripheral blood (Hemoglobin and the total number of white blood cells and the numbers of eosinophils) .

Eosinophile cell / mm^3 Mean ±SD	WBC cell / mm^3 Mean ±SD	Hb g/100 ml Mean ±SD	Parasite No. of infection
116.9 ±104.25	8996 ±5167.61	10.9 ±1.55	*E. histolytica* *N*=288
231.0 ±142.02	7700 ±6673.08	9.7 ±1.65	*G.lamblia* *N*=24
112 ±95.45	5600 ±1767.76	10.9 ±2.12	*E. vermicularis* *N*=5
310 ±0.00	6200 ±0.00	10.5 ±0.00	*H. nana* *N*=1
180 ±0.00	6000 ±0.00	13.4 ±0.00	*T.hominis* *N*=1
236.0 ±0.00	5900 ±0.00	11.6 ±0.00	*E .coli* *N*=1
130 ±80.82	13000 ±10392	11.5 ±1.41	*E. histolytica + G. lamblia* *N*=10
540 ±0.00	9000 ±0.00	8.3 ±0.00	*E.histolytica + H. nana* *N*=1
456.0 ±0.00	7600 ±0.00	11 ±0.00	*G.lamblia + H.nana* *N*=1
0 ±0.00	4500 ±0.00	11.3 ±0.00	*E.histolytica +G. lamblia +T. hominis* *N*=1

T tabulated =1.83,T calculated for Hb = 26.18,T calculated for T.W.B.C = 9.62
T calculated for Number of eosino= 4.42

Table (2) The impact of infection *E.histolytica* of some of the criteria for red blood cell

MCV (vemtoliter)	RDW	Hematokrit Hct %	Hemoglobin Hb (G / ml 100)	MCH (pg)	MCHC (G / ml 100)	Red blood cell count x^6 10/ mm^3 RBCs	Criteria for blood Infection
74.15 ± 2.59	13.63 ± 0.50	36.25 ± 1.13	11.74 ± 0.37	26.47 ± 1.06	35.67 ± 0.20	5.04 0.15±	Infected
69.16 10.31±	13.23 ±0.65	36.21 ± 1.89	11.73 ± 0.62	28.01 ± 0.70	35.81 0.32±	4.63 0.20±	Non infected
0.53	0.49	0.016	0.02	1.12	0.38	1.65	T calculated

P< 0.05 ,T tabulated = 1.78

Table (3) The impact of sex on some of the criteria for red blood cells of people with *E.histolytica*

MCV (vemtoliter)	RDW	Hematokrit Hct %	Hemoglobin Hb (G / ml 100)	MCH (pg)	MCHC (G / ml100)	Red blood cell count x^6 10/ mm^3 RBCs	Criteria for blood Infection
72.32 ± 1.98	14.05 ± 0.47	35.12 ± 2.0	11.37 ± 0.66	25.6 ± 0.89	35.37 ± 0.27	5.12 ± 0.24	Male
75.97 5.02±	13.22 ± 0.93	37.37 1.04±	12.12 ± 0.34	27.35 ± 1.98	35.97 0.23 ±	4.95 ± 0.21	Female
0.67	0.79	0.99	0.99	0.80	1.64	0.496	T calculated

P<0.05 ,T tabulated = 1.89

Table (4) The rate of concentration of IgG in children with sero-parasite *E.histolytica*

Standard error	Standard deviation	Average	Range	Number	The group
86.81	513.60	1024.19	2115.50	35	Infected
57.90	258.95	990.29	1014.30	20	Control

P< 0.05 T calculated = 0.27 T tabulated = 1.67

Table (5) Rate of IgM concentration in children with sero-parasite *E.histolytica*

Standard error	Standard deviation	Average	Range	Number	The group
12.51	55.97	116.78	207.30	20	Infected
64.38	287.91	951.38	1014.30	20	Control

P< 0.05 T calculated = 12.72 T tabulated =1.68

Discussion

The present study showed that included five hospitals to cover different parts of the province of Thi-Qar with total infection 33.3% which less than [20, 21] and approach [22, 23] and higher than [24, 25] .

Differences and similarities in the results may be due to the different regions and years, climatic conditions and the economic, social or cultural experience in the accuracy of screening where a lot of material in the feces suspected of being parasites and are only materials execratory non-digestible or they parasites and is believed to be droplets of fat or otherwise, as well as the method used in the examination was only uses the direct method does not use methods other concentration (sedimentation or flotation) in the examination of the stool .

Effect of type and the type of parasite infection on some blood components .

The present study showed presence of significant differences for the effect of type of infection and the type of parasite on some components of the blood showed the highest value of Hb found at the infection of *T. hominis* with 13.4 while the lowest value the infection occurred with (*E.histolytica* + *H. nana*) and was 8.3 .

The explanation is that the pair infection of by (*E.histolytica* + *H. nana*)

affect the multiplier effect in the participation of the host diet compared to the single infection especially as abdominal pain and diarrhea are the main symptoms of *H. nana* is added to the diarrhea caused by a parasite *E.histolytica* According to the definition of [26] for diarrhea it causes loss of fluid and electrolyte loss and therefore, the recent addition to the flow of blood with the faeces caused by parasitic *E.histolytica* leads to the decrease in the PCV and the occurrence of anemia.

The mutant of *T. hominis* is deemed the non-pathogenic parasites and are therefore less stressful than the rest of the parasites under study. Recorded the highest number of white blood cells, where the incidence of (*E. histolytica* + *G. lamblia*) The lowest number was found in the case of triple infection (*E.histolytica* + *G. lamblia* + *T. hominis*) and with number 4500 .

The total number of white blood cells increase or decrease away from the normal limit for it because i represents the way the defense of the body occurs this disorder as a result of the nomination of the cell of immune cells Basophils and lymphocytes in areas of infection (27) or the crash of neutrophils resulting from the factors of virulence of the parasite [28].

The highest number of eosinophils was 540 at the infection of (*E.histolytica* + *H. nana*), while not recorded infection of triple with protozoa (*E.histolytica* + *G. lamblia* + *T. hominis*) any number and the reason is due to the presence of eosinophiles associated with the worms more than protozoa also decrease the total number of white blood cells with triple infection may be due to protozoa recent infection with these organisms.Impact of *E.histolytica* on some criteria of red blood cell

The difference disappearance between infected and non-infected patients according some criteria bloody addressed in the study, and this result disagreement with [29,30,31] .

On the other hand we did not find any effect of the sex on the criteria for red blood cells (RBCs, Hb, MCH, MCHC, Hct, MCV, RDW) for children which is agreement with [31] . The reason is attributable not to make this parasite severe intestinal bleeding can be caused by hookworm also infection may not be chronic for children handlers in the study.

It should be indecate that humoral immunity plays a role in the immune response against the parasite *E.histolytica* and that was less than the role of cellular immunity against the parasite [32]. In the present study did not find significant differences in IgG concentration between patients and non-infected therefore our result agreement with [21] and disagreement with [33,34]. Significant differences have found in the concentration of IgM between infected and non infected and that does not comply with [21,27,33,34]. The reason as stated on the IgG and the IgM may be due to the novelty of parasite infection and the weakened immunity system in children.

Was reported [35] that the increase in IgM occur in the first three months of infection, IgG remains for several years after infection .

The parasite *E. histolytica* most commonly because it move directly it does not need to intermediate host, criteria of blood is difference in affect according to the type of parasite, there are parasites are not affected by the criteria of blood such protozoa . concentration of IgG does not change only in chronic cases, the lack concentration of IgM may indicate the lack of immune the children concerned .

Appendix shows the abbreviations

Hct Hematokrit
RBCs Red Blood Corpuscles count
Hb Hemoglobin
MCH Mean Corpuscular Hemoglobin
MCHC Mean Corpuscular Hemoglobin Concentration
MCV Mean Corpuscular Volume
RDW Red cell Distribution Width

Reference

1- WHO. (2010). Intestinal parasites . Available at : http : // Apps. Who. Int /ctd/ intpara /burdens.htm.

2- Dariel J . (2007). Cleanse and Purify Thyself Book One. Medford , Oregon Christobe publishing .

3- Choi , M .H ., Sajed ,D., Poole, L.,; Hirata,K; Herdman, S.;Torian, B.E. and Reed, S. L.(2005). An unusual surface protects invasive *Entamoeba histolytica* from oxidant attack. www.sciencedirect.com

4- Razmjou, E. ; Rezaian, M. ; Haghighi, A. ; Kazemi, B. ; Farzami, B.; Kobayashi, S. & Nozaki, T. (2005). Comparison of the recombinant glucose phosphate isomerase from different zymodems of *Entamoeba histolytica* with their natural counterparts by isoenzyme electrophoresis. Iranian . J. Publ. health ., 34(4) : 35 – 40.

5- Frey , R.J.(2006) . Amoebiasis . Gale Encyclopedia of Medicine Gale Group.

6- Mengistu, A.;Gebre- Selassie, S. & Kassa, T. (2007). Prevalence of intestinal parasitic infections among urban dwellers in Southwest Ethiopia . [Ethiop. J. Health Dev., 21 (1) : 12 - 17]

7- I.N.A.C.G.(1997). Guidelines for eradication of iron deficiency anemia. A report of the International Nutritional Anemia Consultative Group (INACG) . New York, Washington, Nutrion foundation .

8 - Crompton, D. (2000). The Public health importance of hook worm disease. Parasitology, 121: 39 - 50.

9- Stephenon, L.S. ; Holland, C.D. & Cooper, E.S. (2000). The public health significance of *Trichuris trichiura*. Parasitology, 121 : 73 –95.

10- Kasper, L .H. and Buzoni – Gatel, D. (2001) . Up and downs of mucosal cellular immunity against protozoan parasite. Infect. Immun. 69 (1): 1-8.

11- Ichhpujani, R.L. & Bhatia,R. (1994) . Medical Parasitology, 1st edu. Jaypee Bros. Med. Puble, New Delhi : 384 pp.

12- Paniker, C. J .(2007) . Textbook of Medical Parasitology. 6th Edition . India . Jaypee Brothers Medical Publishers .

13- Lumsden, W.H., Burns, S. And McMillan, A. (1996) .Protozoa in practical medical microbiology. By Collee, J.G., Marmion, B.P., Fraser, A.G and Simmons , A. Churchill Living stone, Tokyo .pp. 741 -742 .

14- Zeibig , E.A. (1997) . Clinical parasitology: A practical approach . W.B. Saunders Company , Philadelphia : 320 pp.

15- Jubouri, Talal Hamad Ali Musa (2008). Epidemiological study of patients with clinical and Giardia Giardiasis in children Shirqat city and its suburbs, Master Thesis, Faculty of Education, University of Tikrit.

16- Harmening, D. M. (2009). Clinical Hematology and Fundamentals of Hemostasis . fifth edition . United States of America F. A. Davis Company. Philadelphia .

17- Ahamid A.S.& Salhi, E.M.(1994). Practical physiology for community Health departments. Ministry of Higher Educ. &Scientific Research, foundation of Technical institutes. AL-Mosul Univ. Press.

18- Talib, V.H. & Khurana, S.R. (1996) . A handbook of medical laboratory technology . 5th , edu., C.B.S. publ., New Delhi .

19- Lewis, S.M. ; Bain, B.J. and Bates, I. (2001) . Dacie and Lewis Practicle Hematology . 9th ed. Churchill Livingstone .

20- Akhalifawi, Mohammad Jubair (2006). Study of the parasites of the gastrointestinal tract in children under five and their impact on some of the blood levels: Master – Faculty of Science - University of Mustansiriya: 92

21- AL-Rukabi, Nuha Jabbar (2006). A study of some intestinal parasites that cause diarrhea in children in the city of Nasiriyah. Master Thesis. Faculty of Education - University of Thi -Qar. 92 .

22-Wadood, U.A. ; Bari, A. ; Rhman, A.U. & Qasim K. F. (2005). Frequency of Intestinal Parasite Infestation in Children Hospital Quetta . Pakistan J. Med . Res., 44(2).

23- Al-Dulaimi, Khadija Abdullah Khalif (2001). Epidemiological study in the parasite Entamoeba histolytica amoebic dysentery city of Ramadi. Master Thesis, Faculty of Science, University of Anbar: 74 .

24- Chandrashekhar ,T.S.; Joshi, H.S.; Gurung, M.; Subba, S.H.; Rana, M.S. and Shivananda, P.G .(2005) . Prevalence and distribution of intestinal parasitic infections among school

children in Kaski District, Western Nepal . JMBR : A peer –review Journal of Biomedical Sciences Vol. 4 No. 1 pp -78 -82.

25- Zakai, H.A.(2004). Intestinal parasitic infections among primary school children in Jeddah ,Saudi Arabia. Journal of the Egyptian society of parasitology, 34(3) : 783 -790 .

26- Pickering, L. K. and Snyder, J.D. (1996). Gastroenteritis. In: Behrman, R.E. ; Kliegman, R. M. and Arvin, A.M. (Eds.). Nelson textbook of pediatrics, 15th ed., W.B. Saunders, Philadelphia : 721 – 724

27- Al – Kubassi , A. B. H. (2002). Immunological, epidemiological study of patients infected with *Entamoeba histolytica* . Ph.D. Thesis . Coll. Sci. Al – Mustansiriya Univ.

28- Que, X. and Reed, S. L. (2000) . Cysteine proteinases and the pathogenesis of amebiasis . Clin. Microbiol. Rev. 13(2): 196 -206.

29- Hopkins , R. M.; Gricey, M.S. ; Hobbs, R. P. ; Spargo , R.M.; Yates , M. & Thompson R.C.A. (1997) . The prevalence of hookworm infection , iron deficiency and anemia in an aboriginal community in north – west Australia. M. J. A., 166: 241 – 244

30- AL-Nahi, Alaa Shaker (1998). Epidemiological study of intestinal parasites among primary school students in the Najaf area. Master Thesis - Faculty of Education - University of Kufa: p. 87.

31- Al-Zubaidi, Zainab Hadi (2002). Worm hookworm Ancylostoma duodenale and its impact on the image Mhimh blood in the village in the province of Babylon. Master Thesis. Faculty of Science - University of Babylon.

32- Campbell , J.D. and Chadee , K . (1997) . Interleukin IL – 2 , IL – 4 and tumor necrosis factor – alpha responses during *Entamoeba histolytica* liver abscess development in gerbils . J. Infect. Dis. 175(5): 1176 – 1183 .

33- Arianpour, N. and Mohapatra , T.M. (2003) . Study of antiamoebic antibody in amoebiasis using ELISA and RID techniques . Iranian J. Publ. Health , 32(4): 13 – 18 .

34- Hafiz, Alia Abdul Al- Hussein. (2007). Study the immune response, cellular and humoral in patients with intestinal amebiasis, Master. Faculty of Science - University of Basra. P. 64.

35-Valenzuela , O. ; Ramos , F. ; Moran, P. ; Gonzalez, E.; Naladez, A.; Gomez , A.; Melendro , E. I. ; Ramiro , M. ; Munoz , O. and Ximenez, C. (2001) . Persistense of secretory antiamoebic antibodies in patient with past invasive intestinal or hepatic amoebiasis . Parasitol. Res. 87 (10) : 849 – 852 .