EOSINOPHILIA AND INTESTINAL HELMINTHIASIS AMONG SCHOOLCHILDREN IN WADI DHAHR DISTRICT, SANA'A GOVERNORATE, YEMEN

Abstract

Objectives: Allergy and parasitic infections are common causes of blood eosinophilia. Intestinal helminthiasis remains a major health problem in many developing countries. Eosinophils are effector immune cells against parasites.The study illustrates the prevalence of eosinophilia andintestinal helminthiasis among primary schoolchildren in WadiDhahr district, Sana'a governorate, Yemen.

Subjects and methods: Four hundred and fourteen pupils were enrolled in this study. Blood and stool samples were collected from each pupil. Blood smears were stained with Giemsa stain and used for counting the eosinophils' percentages. Stool samples were examined using Hoffman's sedimentation method for presence of worms' ova.

Results: Eosinophilia was found in 134 (32%) of pupils; 86 (30%) males and 48 (37%) females. Infection with intestinal helminths occurred in 79 (19%) of students; 52 (18%) males and 27 (21%) females. Hymenolepiasis was the most common intestinal worms among schoolchildren (44; 10.6%). Eosinophilia was common among school children infected with intestinal worms ($\chi 2 = 32.8$, $p < 0.001$). Eosinophilia was more frequent among children infected with *Ascarislumbricoides*,*Hymenolepis nana* and *Enterobiusvermicularis*than other intestinal worms ($\chi 2 = 20.1$, $p<0.001$; $\chi 2 = 16.1$, $p<0.001$; $\chi 2 =8.4$, $p = 0.007$) respectively.

Conclusions: Eosinophilia was common among schoolchildren and was strongly associated with ascariasis,hymenolepiasisand enterobiasisbut not with taeniasis and trichuriasis.

Keywords

Eosinophilia, intestinal helminthiasis, schoolchildren, WadiDhar, Yemen

INTRODUCTION

Typically, eosinophils in the blood are often less than 6% of blood leukocytes. Varied disorders and etiologies may increase blood eosinophils. Common causes of eosinophilia include helminthic infections and allergic diseases. Parasitic infections are the most common cause ofpersistent eosinophilia in developing countries while atopic diseases such as eczema and asthma are the most common causes in developed countries[1,2]. In some parasitic infections, eosinophilia may be the only symptom[3]. Eosinophilia may vary in relation to the parasite development stage, parasite location in the body, parasite load and the co-infections with more than one parasite. Infections by helminths with life cyclesthat include tissue migratory phases, such as trichinosis and ascariasis, induce sustained elevatedeosinophilia in host blood and tissues[4,5].

Globally 1.5 billion individuals were infected with intestinal helminths; of which over 267 million pre-school children and over 568 million school children are considered at risk of morbidity, particularly developing countries[6]. In 2010, anestimated 819 million people worldwide were infected with *Ascarislumbricoides*, 464 million with *Trichuristrichura*, and 438 million with hookworm[7].

Helminthic infections can cause significant nutritional deficiencies, delayed physical and cognitive development during childhood and reduced productivity in adults[8-10]. These infections have been associated with poor personal hygiene, environmental sanitation and limited access to potable water[11].

In Yemen, intestinal helminth infections are common particularly in rural communities. Two studies performed by Al-Mekhlafi*et al*, 2016,reported the overall prevalence of intestinal parasitic infections among school children to be 17.2 %[12]. Data on the prevalence of eosinophilia and intestinal helminthiasis among schoolchildren are limited. The study aimed to assess the prevalenceof intestinal helminthiasis and its association with eosinophilia among schoolchildren.

SUBJECTS AND METHODS

Study design and area

A cross-sectional survey was conducted during January 2016 to June 2018. The two primary schools (AL-Wahda and AL-Mutanabi) present in WadiDhahr, a rural district in northeast Sana'a where parasitic infections are common were enrolled in this study.

Study population

Total of 414 pupils were examined. Simple random sampling was used to choose participants from each school using the lists of all students in each school. One hundred and ninety six students were selected from AL-Wahda school while 218 students were selected from AL-Mutanabi school.

Exclusion criteria

Students who were taking medications that may affect eosinophilia (e.g. penicillins and cephalosporins) or who had received anti-helminthic drugs within three months from the beginning of the study. Also students known to have food allergy or asthma were excluded from the study.

Ethical considerations

The study was approved by the Faculty of Medicine and Health Sciences, Sana'a University and heads of schools. Oral consent was obtained from pupils' parents to participate in the study before samples collection. School children gave a verbal consent which was approved for children by the ethical committee after their parents' consent.

Sample collection

Two samples were obtained from each participant: blood and stool specimens. Blood samples were collected by finger prick using disposable lancets. Blood smears were made from capillary blood on a glass slide, left to air dry and then fixed with absolute methanol. One gram of stool sample was emulsified in 7ml of 10% formalin for fixation.

Examination of blood films

Differential blood count:

Fixed blood films were stained by Giemsa method[13]. Blood films were washed and left to dry in air. Dried films were then examined microscopically using 40X and 100X objectives. One hundred white blood cells (WBC) were counted to determine the eosinophil percentages in the peripheral blood of each student. Eosinophil count greater than 6% was considered to be eosinophilia.

Examination of stool specimens

Stool samples were examined using Hoffman's sedimentation method [14]. Helminthic ova are concentrated by passing the fecal suspension through a gauze followed by centrifugation for two minutes at 1000 rpm. The upper liquid phase was discarded using a pipette. Two slides per fecal sample were prepared and read by two investigators.

Statistical analysis

All statistical analyses were performed using SPSS, version 20. Data are presented as numbers and percentages. The statistical analysis was performed using a Pearson correlation to determine the association of eosinophilia helminths infection. *p* value less than 0.05 indicated statistical significance.

RESULTS

A total of 414 school children from four schools were enrolled in this study. Among the study participants, 285 (69%) were males and 129 (31%) were females. Their age ranges from 5-15 years old with mean age 12±1.7 years old. They were grouped into two age-groups. The first group included 67(16%) students aged 5-10 years. The second group contained 347(84%) students aged 11-15 years, table 1.Eosinophilia was found in 134 (32%) of students; 86 (30%) males and 48 (37%) females. Infection with intestinal helminths occurred in 79 (19%) of students; 52 (18%) males and 27 (21%) females, table 1.

Eosinophilia was more frequent among students of age group 5-10 years than older students with statistically significant difference (χ^2 = 10.4, p = 0.002). It was also higher among students infected with intestinal parasites (47; 60%) than in non-infected students (87; 26%). This difference was statistical significance (χ^2 = 32.8, p< 0.001). No statistical difference was found between eosinophilia among males and females, table 2.

Hymenolepiasis was the most common intestinal worms among schoolchildren (44; 10.6%), followed by ascariasis (17; 4.1%),enterobiasis (11; 2.7%) and taeniasis (10; 2.4%). Ten (2.4%)students had mixed worm infections, i.e. infected with more than on intestinal helminths, table 3.

Eosinophilia was found in 26 (59%) of students infected with *Hymenolepis nana*, 14 (82%) students infected with *Ascarislumbricoides*, 8 (73%) students infected with *Enterobiusvermicularis*. Eosinophilia was statistically significant among students infected with *Ascarislumbricoides*(χ^2 = 20.2, p< 0.001), *Hymenolepis nana* (χ^2 = 16.1, p< 0.001), *Enterobiusvermicularis* (χ^2 = 8.4, P = 0.007), table 4. However, eosinophilia was not significant among pupils infected with *Taeniasaginata* (χ^2 = 0.03, P = 0.57), *Trichuristrichiura* (χ^2 = 3.3, P = 0.089), table 4. Eosinophilia was found in all pupils who were infected with more than one intestinal helminth.

DISCUSSION

Intestinal parasitic infections are still major public health problems in developing countries and affect the poorest and most deprived communities. Soil-transmitted helminths impair the nutritional status and affect general intelligence of the people they infect. Malnutrition has a significant impact on growth and physical development of the infected children[6,15].

Our study confirms intestinal helminthiasis to remain a problem in children from the two elementary schools at WadiDhahr district with prevalence of 19%.The high prevalence of intestinal helminthiasismay reflects poor adhesion to preventive measures which helps re-infection to occur after dewormed programs performed by WHO. Hymenolepiasis was the most frequent intestinal helminthiasis among schoolchildren followed by ascariasis and enterobiasis.Higher prevalence of hymenolepiasisin the study population may be attributed to the easily mode oftransmission either by autoinfection or from person to person without requiring an intermediate host[16].

Eosinophilia was common among school children infected with intestinal worms. This could be explained by the fact that worm infections induce immune responses via T helper cells type 2 subset (T_H2 cells). T_H2 cells produce interleukin-4 (IL-4), IL-5, IL-10and IL-13 which stimulate more production of eosinophils from bone marrow resulting in peripheral blood eosinophilia[17-21]. Presence of blood eosinophilia without intestinal helminthiasiscould be attributed to allergies, other worm infection such as urinary schistosomiasis or light number of parasites in the students' gastrointestinal tracts.

Eosinophilia was more frequent among children infected with *Ascarislumbricoides*than other intestinal worms. This could be explained by presence of tissue-invading larvae which migrate from small intestine into blood circulation to reach lungs and elicit pulmonary inflammation[4,22-24]. Nevertheless, eosinophilia was non-significant among schoolchildren infected with *Trichuristrichiura* and *Taeniasaginata*. This may be interpreted by that worms that do not invade host tissues and thus do not come in contact with host immune system. Similar observationswere reported by other studies[25-27].

Limitations for this study wasneither availability of nearby laboratory to perform complete blood count in order to calculate absolute eosinophil countsnorrefrigerator for sample storage till they were being investigated.

CONCLUSION AND RECOMMENDATION

Our study indicates that eosinophil percentages for schoolchildren who were infected with intestinal helminthiasis were significantly higher than in schoolchildren who were not infected.

ACKNOWLEDGEMENTS

Authors are grateful to all students and teachers who participated in this study.

CONFLICT OF INTEREST

There is no conflict of interest related to this work

AUTHORS' CONTRIBUTIONS

Authors contributed equally to the design, implementation, statistical analysis and manuscript drafting. All authors read and approved the final manuscript.

References

1. Rothenberg M.Eosinophilia. N Engl J Med.1998;338(22):1592-1600.
2. Kovalszki A, Weller PF.Eosinophilia. Prim Care.2016;43(4):607–617.
3. Ardiç N. An overview of Strongyloidesstercoralis and its infections. MikrobiyolBul.2009;43:169-77.
4. Nutman TB.Evaluation and differential diagnosis of marked, persistent eosinophilia. Immunol Allergy Clin North Am.2007;27:529-549.
5. Chinchilla RH.Eosinofilia y parasitosis. Rev Med Costa Rica y Centroamerica.2010;593:241-244.
6. WHO.Soil-transmitted helminth infections; 2020;https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections.Access 17June 2020.
7. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ.Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasites and Vectors.2014;21(7):37.
8. Hotez PJ, Bundy DP, Beegle K, *et al*.Disease control priorities in developing countries. Helminth infections: soil-transmitted helminth infections and schistosomiasis. Oxford University Press,Washington, 2006.
9. Papier K, Williams GM, Luceres-Catubig R, *et al*. Childhood malnutrition and parasitic helminth interactions. Clin Infect Dis. 2014;59(2):234-43.
10. Assoum M.The effects of helminth infections on child physical and cognitive development: an integrated pathophysiological and socioeconomic approach.Advances in Life Science and Medicine.2015;1(1):1-23.
11. Gelaw A, Anagaw B, Nigussie B, Silesh B, Yirga A, Alem M, Endris M, Gelaw B.Prevalence of intestinal parasitic infections and risk factors among schoolchildren at the University of Gondar Community School, Northwest Ethiopia: a cross-sectional study. BMC Public Health.2013; 3:304.
12. Al-Mekhlafi AM, Abdul-Ghani R, Al-Eryani SM, Saif-Ali R, Mahdy MA.School-based prevalence of intestinal parasitic infections and associated risk factors in rural communities of Sana'a, Yemen. Acta Trop.2016; 163:135- 141.
13. Bain B, Lewis M.Preparation and staining methods for blood and bone marrow films In: Dacie and Lewis (ed) Practical Haematology. 10thedn, Philadelphia, Churchill Livingstone, 2006, pp59-77.
14. Hoffman WA, Pons JA, Janer JL.The sedimentation-concentration method in schistosomiasismansoni. Puerto Rican Journal of Public Health and Tropical Medicine 1934;2:283-298.
15. Jardim-Botelho A, Raff S, Rodrigues Rde A, Hoffman HJ, Diemert DJ, Corrêa-Oliveira R, Bethony JM, Gazzinelli MF.Hookworm, Ascarislumbricoides infection and polyparasitism associated with poor cognitive performance in Brazilian schoolchildren. Trop Med Int Health. 2008; 13(8):994-1004.
16. CDC.Hymenolepiasis. Reviewed: December 13, 2017. https://www.cdc.gov/dpdx/hymenolepiasis/index.html. Access 14 August 2019
17. Moreau E, Chauvin A.Immunity against helminths: interactions with the host and the intercurrent infections. Journal of Biomedicine and Biotechnology. 2010;2010:428593.
18. Shamri R, Xenakis JJ, Spencer LA.Eosinophils in innate immunity: an evolving story. Cell Tissue Res. 2011; 343(1):57–83.
19. Alcântara-Neves M, Britto1G, Veiga R, Figueiredo C,Fiaccone R, Conceição Jackson, *et al*.Effects of helminth co-infections on atopy, asthma and cytokine production in children living in a poor urban area in Latin America. BMC Res Notes. 2014;7:817.
20. Weatherhead JE, Hotez JP. Worm infections in children. Pediatrics in Review. 2015;36(8):341-354.
21. Kumar S, Jeong Y, Ashraf MU, Bae YS.Dendritic cell-mediated Th2 immunity and immune disorders. International journal of molecular sciences. 2019;20:2159-2186.
22. Shin MH, Lee YA, Min DY.Eosinophil-mediated tissue inflammatory responses in helminth infection. Korean J Parasitol. 2009;47Suppl:S125- S131.
23. Mejia R, Nutman T.Evaluation and differential diagnosis of marked, persistent

Eosinophilia. SeminHematol. 2012;49(2):149–159.
24. Curtis C, Ogbogu PU.Evaluation and Differential Diagnosis of Persistent Marked Eosinophilia. Immunol Allergy Clin North Am. 2015;35(3):387-402.
25. Sumagaysay JB, Emverda FM.Eosinophilia and incidence of soil-transmitted helminthic infections of secondary students of an indigenous school. Asian Journal of Health. 2011;1 (1):172-184.
26. De-Farias S V,Silva E, Medeiros S, Souza L, Silva V, Brito T,Machado P.Serum total IgE, *Ascarislumbricoides* specific IgE and eosinophils in parasites-infected children in a tropical area. World Allergy Organization Journal. 2015;8 Suppl 1:A223.
27. Jiero S, Ali M, Pasaribu S, Pasaribu A.Correlation between eosinophil count and soil-transmitted helminth infection in children. Asian Pacific Journal of Tropical Disease. 2015; 5(10):813-816.

Table 1: Characteristics of schoolchildren participate in the study

	Males (n = 285)		**Females (n = 129)**		**Total**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Age groups						
5-10 years	45	16	22	17	67	16
11-15 years	240	84	107	82	347	84
Mean age ± SD*	12±1.7					
Eosinophilia						
Yes	86	30	48	37	134	32
No	199	70	81	63	280	68
Intestinal helminths						
Infected	52	18	27	21	79	19
Non-infected	233	82	102	79	335	81
Total					414	100

*SD: standard deviation

Table 2: Eosinophilia among students according to their gender, age groups and Intestinal helminthiasis

* *P* value is significant (≤ 0.05)

	Number	%
Hymenolepiasis	44	10.6
Ascariasis	17	4.1
Enterobiasis	11	2.7
Taeniasis	10	2.4
Trichuriasis	6	1.4
Schistosomiasis	2	0.5
Mixed infection	10	2.4

Table 3: Types of worm infections among schoolchildren

Species of worms	**Eosinophilia**		**Normal**		χ^2	***C.I***	***P* value**
	No.	**%**	**No.**	**%**			
Ascarislumbricoides	14	82	3	18	20.2	0.026-0.329	< 0.001*
Hymenolepis nana	26	59	18	41	16.1	0.150-0.542	< 0.001*
Enterobiusvermicularis	8	73	3	27	8.4	0.045-0.654	0.007*
Trichuristrichiura	4	67	2	33	3.3	0.042-1.293	0.089
Taeniasaginata	3	30	7	70	0.03	0.285-4.399	0.59

Table 4: Association of eosinophilia with different types of intestinal worms

	Eosinophilia		**Normal**		**OR**	***C.I***	χ^2	***P* value**
	NO.	**%**	**NO.**	**%**				
Age groups								
5-10	33	49	34	51	2.4	1.39-4.02	10.4	0.002*
11-15	101	29	246	71				
Intestinal helminths								
Infected	47	60	32	40	4.2	2.5-6.98	32.8	< 0.001*
Non-infected	87	26	248	74				
Gender								
Males	86	30	199	70	1.4	0.89-2.12	2	0.17
Females	48	37	81	63				