**Reviewer’s Comments**

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**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 the 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 (χ2 = 32.8,*P*<0.001). Eosinophilia was more frequent among children infected with *Ascarislumbricoides*,*Hymenolepis nana* and *Enterobiusvermicularis*than other intestinal worms(χ2= 20.1, *P*<0.001; χ2= 16.1, *P*<0.001;χ2=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:**

**INTRODUCTION**

Typically, eosinophilscount in the blood are often less than 6% of the total leukocyte count. Increase in the eosinophil number more than 6% will cause eosinophilia. 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 countries1,2. In some parasitic infections, increase in the eosinophilia number may be the only symptom3.

Eosinophilia may vary in relation to the parasite development stage, parasite location in the body, parasite load, and 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 tissues4,5.

Globally 1.5 billion individuals were infected with intestinal helminths; of which over 267 million pre-school children and over 568 million schoolchildren are considered at risk of morbidity, particularly developing countries6. In 2010,anestimated 819 million people worldwide were infected with *Ascarislumbricoides*, 464 million with *Trichuristrichura*,and 438 million with hookworm7.

Helminthic infections can cause significant nutritional deficiencies, delayed physical and cognitive development during childhood and reduced productivity in adults8-10. These infections have been associated with poor personal hygiene, environmental sanitation and limited access to potable water11particularly in Yemen where the most of people live under poverty line that lack the effectively programs for parasitic infection control and prevention12,13,14. It was reported that the prevalence rate of intestinal parasitic infection was 62.7% recorded among schoolchildren in Ibb city13. Also, a study by Al-Mekhlafi*etal*,14reported that the overall prevalence of intestinal parasitic infections was 17.2 % among schoolchildren.

In Yemen, intestinal helminth infections are common particularly in rural communities. Two studies performed by Al-Mekhlafi*etal*, 2016,reported the overall prevalence of intestinal parasitic infections among school children to be 17.2 %12.In Yemen, there are several investigations that focused on the prevalence of intestinal parasitic infection among children school and associated with environment factors. 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.

 **MATERILESAND 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 a food allergy or asthma were excluded from the study.

**Ethical considerations**

~~The~~This 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 7mLof 10% formalin for fixation.

**Examination of blood films**

**Differential blood count:**

Fixed blood films were stained by Giemsa method13. 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 (χ2 = 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 (χ2 = 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*(χ2 = 20.2, *P*< 0.001), *Hymenolepis nana* (χ2 = 16.1,  *P*< 0.001), *Enterobiusvermicularis* (χ2 = 8.4, *P* = 0.007), table 4. However, eosinophilia was not significant among pupils infected with *Taeniasaginata* (χ2 = 0.03, *P* = 0.57), *Trichuristrichiura* (χ2 = 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 the general intelligence of the people they infect. Malnutrition has a significant impact on the growth and physical development of the infected children6,15.

Our study confirms intestinal helminthiasis to remain a problem in children from the two elementary schools at WadiDhahr district with a prevalence of 19%.The high prevalence of intestinal helminthiasismay reflects poor adhesion to preventive measures which helpre-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 easy mode oftransmission either by autoinfection or from person to person without requiring an intermediate host16.

Eosinophilia was common among school children infected with intestinal worms. This could be explained by the fact that worm infections induce immune responses viaThelpercell type 2 subset ( Th2cells). Th2cells produce interleukin-4 (IL-4), IL-5, IL-10,and IL-13 which stimulate more production of eosinophils from bone marrow resulting in peripheral blood eosinophilia17-21. The presence of blood eosinophilia without intestinal helminthiasiscould be attributed to allergies, other worm infections such as urinary schistosomiasis or a 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 the presence of tissue-invading larvae thatmigrate from the small intestine into blood circulation to reach lungs and elicit pulmonary inflammation4,22-24. Nevertheless, eosinophilia was non-significant among schoolchildren infected with *Trichuristrichiura* and *Taeniasaginata*. This may be interpreted by worms that do not invade host tissues and thus do not come in contact with the host immune system. Similar observationswere reported by other studies25-27.

Limitations for this study wasneither availability of nearby laboratory to perform complete blood count in order to calculate absolute eosinophil countsnorrefrigeratorfor sample storage untill 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.

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**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.

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**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 |
| **Intestinal helminths** |
| Infected | 52 | 18 | 27 | 21 | 79 | 19 |
| Non-infected | 233 | 82 | 102 | 79 | 335 | 81 |
| **Eosinophilia** |
|  Yes | 86 | 30 | 48 | 37 | 134 | 32 |
|  No | 199 | 70 | 81 | 63 | 280 | 68 |
| **Total** | 414 | 100 |

\*SD: standard deviation

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Eosinophilia** | **Normal** | **OR** | ***C.I*** | **χ2** | ***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 |

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

\* *P* value is significant (≤ 0.05)

**Table 3: Types of worm infections among schoolchildren**

|  |  |  |
| --- | --- | --- |
| Parasitic disease  | 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 4:** **Association of eosinophilia with different types of intestinal worms**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Species of worms** | **Eosinophilia** | **Normal** | **χ2** | ***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 |

\* *P* value is significant