Research Article

Determinant factors associated with parasitic infestation among pediatric cases with unexplained eosinophilia

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ABSTRACT

Background: In tropical countries like India, common causes of eosinophilia are parasitic infestation, unhygienic living condition, and poor sanitation that accounts for high worm infestation in India. **Objective:** The present study was aimed to study the association between various risk factors and parasitic infestation in pediatric cases with unexplained eosinophilia. **Materials and Methods:** The study population comprised of 384 children. A well predesigned and pre-tested questionnaire was used to collect detailed related history and socio-demographic details. Saline and iodine wet mount of stool sample was made to detect intestinal parasites. For detection of microfilaria, Leishman's staining of peripheral blood smear was done. Finally, the statistical analysis of the collected data was carried out. **Results:** Out of 384 participants, 7.03% of the total population was found to be positive for intestinal parasites. None of the peripheral blood smears showed the presence of microfilaria. Most of the positive study participants were from low or middle socio-economic sectors. Very few parents were graduates. 6.5% of children in this category were found to show parasites in their stool samples. Hygiene status, hand washing, and stool disposal habits of patients were found to be significantly associated with the presence of the parasite. **Conclusion:** Possible socio-demographic, environmental and behavioral factors associated with parasitic infestation among pediatric cases with unexplained eosinophilia need to be studied to implement effective control measures so as to provide better health care and health benefits to the children.

KEY WORDS: Eosinophilia; Socio-demographic; Parasitic Infestation; Pediatric; Factors

INTRODUCTION

Eosinophils are a prominent aspect of numerous parasitic diseases. The pattern and degree of eosinophilia in parasitic infections are determined by the development, migration, maturation, burden, and distribution of the parasite within the host as well as by the host's immune response. Parasites tend to provoke marked eosinophilia when they or their products interact with immune effect or cells in tissues, chiefly during migration (e.g., trichinosis, ascariasis, gnathostomiasis, and filarial parasites). Provocation of eosinophils in the blood is usually absent when there is a mechanical hurdle between the parasite and the host (e.g., adult tapeworms that are solely intraluminal or hydatid cysts that are enclosed in a cystic structure).[1,2] In tropical countries like India, the most common cause of eosinophilia is a parasitic infestation, unhygienic living condition, and poor sanitation that accounts for high worm infestation in India.[3] Definitions of hypereosinophilia and the hypereosinophilic syndrome are based on the proposal by Chusid *et al*.[4] of an eosinophil count of 1.5 × 109-109

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or greater persisting for at least 6 months, for which no underlying cause can be found and which is associated with signs of organ involvement and dysfunction. This criterion was subsequently accepted in the World Health Organization classification of chronic eosinophilic leukemia.[5] Primary and idiopathic eosinophil disorders are rare and probably under-diagnosed conditions. A large population-based study in a general practice setting from Copenhagen demonstrated an incidence of eosinophilia (defined as a count of at least 0.5 × 109-109) of 4%.[6] The high prevalence of intestinal parasitic infection is closely associated with poverty, climatic conditions, poor personal hygiene, poor sanitation, and unsafe drinking water.[7-10] The significance of the association of high absolute eosinophilia with non-helminthic parasitic diseases is varied. Non-helminthic parasites such as protozoans, flagellates, apicomplexans, coccidian parasites, and ciliates pose increased risk to patients in terms of morbidity such as diarrhea, malabsorption, and abdominal discomfort and high mortality in untreated cases. Therefore, this study was carried out focusing on various factors associated with eosinophilic children harboring intestinal parasites in their stool specimens.

The present study was undertaken to study the association between various possible risk factors favoring parasitic infestation in pediatric cases attending a tertiary care center with unexplained eosinophilia.

MATERIALS AND METHODS

It was a cross-sectional (observational) study done to study the association between various possible determinant factors and parasitic infestation in eosinophilic pediatric cases referred from the Department of Paediatrics to the Department of Microbiology, Integral Institute of Medical Sciences and Research, Lucknow. This study which lasted over a period of 6 months, i.e., January–June 2017 was conducted on 384 subjects in the parasitology section of the microbiology laboratory.

Ethical Consideration

The study was approved by the Institutional Research Committee and the Institutional Ethics Committee.

Inclusion Criteria

Children ≤14 years of age attending indoor and outdoor units of Paediatrics Department, IIMS and R, having peripheral blood eosinophilia which was confirmed by their complete blood count were enrolled in this study.

Exclusion Criteria

Children who suffered from known causes of eosinophilia such as allergies, asthma, atopic dermatitis, malignancies, or immunodeficiency in the past or at the time of the study were excluded from the study.

Children who had received antihelminthic drugs within 3 months of the beginning of study and children whose accompanying guardian did not give consent for their children's participation in the present research work were also excluded from this study.

Procedure of Collection of Data

The patient's attendants were informed about the study to obtain their consent for their children participation in the present research work. A well predesigned and pre-tested structured questionnaire was used to identify environmental, socio-demographic and behavioral factors. The study was done to correlate risk factors and relevance of parasitic infections on the basis of socio-economic status, educational background of parents, locality, hygiene status, hand washing habits, means of stool disposal, food and water consumption, etc.

Specimen Collection for Detection of Intestinal Parasites and Microfilaria

Stool samples were collected from children to detect intestinal parasites and dispatched to the parasitology section of the microbiology laboratory of the institute where it was further processed. For detection of microfilaria, two specimens of blood were collected in ethylenediaminetetraacetic acid vials, one between 10.00 h and 14.00 h, for those microfilaria having a diurnal periodicity and the other collected between 22.00 h and 2.00 h, for those having nocturnal periodicity.

Laboratory Examination

Stool samples were first examined macroscopically for the presence of gross parasitic stages such as adult worms, larvae, and/or segments of tapeworms, followed by microscopic examination by Lugol's iodine and saline wet mount preparations. Saline wet mount was useful for live motile trophozoite forms. Ova of helminths could also be readily seen. Iodine wet mount was used for identification of cysts.

For detection of microfilaria, Knott's concentration technique was implemented to concentrate blood sample and increase sensitivity. Anticoagulated blood (1 ml) was placed in 9 ml of 2% formalin and centrifuged at 500 g for 1 min. The sediment was used to make smear on a slide and dried thoroughly. The smear was covered with the undiluted Leishman's stain. Care was taken not to overflow with excess stain. Preferably just enough number of drops was added to cover the smear. The undiluted Leishman's stain acted both as a fixative and partially stained the smear. Still, since the moisture content

could have varied, it was better to fix the slide in methanol before staining. Twice the volume of pH 6.8 buffered water was added to dilute the Leishman's stain by gently blowing with a straw or using a plastic bulb pipette. Leishman's stain was allowed to remain for 10–12 min. The stain was washed off with clean (or filtered) tap water. The back of the slide was cleaned and dried in a draining rack. The slide was viewed under the microscope for detection of microfilaria.

Statistical Analysis

Data entry and statistical analysis were performed using the Microsoft Excel and SPSS windows version 16.0 software. Tests of significance such as Mann–Whitney U-test and Kruskal–Wallis Test were applied to find out association among categorical variables. $P < 0.05$ was taken as significant.

Table 1: Literacy status of parents/guardians

Literacy status	*n* (%)
Graduate	60 (15.6)
High school	80 (20.8)
Higher secondary	54 (14.1)
Primary	144 (37.5)
Illiterate	46 (12.0)
Total	384 (100)

Table 2: Mean distribution of parasites in participants with respect to the place of residence and socio-economic status

Variables	Mean rank	Test statistics	*P*-value
Place of residence			
Rural	10.57	U=12.21	*P*=0.004
Urban	4.47		
Socio-economic status			
Low	16.64	χ^2=3.00	*P*=0.002
Middle	10.36		
High	6.00		

RESULTS

Out of 384 participants, 223 were males and 161 were females. 27 children were found to show parasites in their stool specimen, i.e., 7.03% of the total population while 357 children were found as negative cases. The most commonly encountered parasite was cysts/trophozoites of *Giardia lamblia*.

Keeping in view of the objective of this study, an attempt has been made to analyze the socio-demographic, environmental, and behavioral factors. The questionnaire pattern which was followed during this study showed that the literacy status of most of the parents/guardians was primary or hardly up to secondary schools. Very few parents were graduates [Table 1].

Out of 384 patients involved in this study, 337 belonged to rural areas, of which 25 (7.4%) children were found to be positive for parasites. Furthermore, 47 patients were from urban areas of nearby, out of which only 2 (4.2%) children were found to harbor parasites. The ratio between total rural and urban children was found to be 7.1:1 and positive rural and positive urban cases were in the ratio of 12.5:1.

Table 2 illustrates the mean distribution of parasites in participants with respect to the place of residence and socio-economic status. The association between place of residence and socio-economic status of patients for parasites was found to be statistically non-significant.

Most of the study participants were from low or middle socio-economic sectors. Out of 384 children who participated in this study, 304 belonged to low socio-economic background. 20 (6.5%) children in this category were found to show parasites in their stool samples. 80 children belonged to middle socio-economic class, of whom 7 (8.8%) were found to harbor parasites. In the present study, none of the children belonged to high socio-economic background. Details are given in Table 3.

Table 3: Distribution of parasites in participants according to their place of residence and socio-economic status

Parasites	Place of residence		Socio-economic status		
	Rural	Urban	Lower	Middle	Upper
G. lamblia	8	0	5	3	0
E. histolytica	5	1	4	2	0
H. nana	6	1	5	2	0
B. hominis	3	0	3	0	0
T. hominis	1	0	1	0	0
E. histolytica and *B. hominis*	1	0	1	0	0
E. histolytica, G. lamblia, and *B. hominis*	1	0	1	0	0
Total	25	2	20	7	0

G. lamblia: Giardia lamblia, E. histolytica: Entamoeba histolytica, B. hominis: Blastocystis hominis, H. nana: Hymenolepis nana, T. hominis: Trichomonas hominis

Table 4 depicts that only 15 children gave a history of food intake other than homemade food which were street foods, market made foods, or fast foods from outdoor. In this category, 6 (40%) children had parasites in their stool samples. Only 2 out of 27 with good hygienic habits harbored parasites. In this study, patients and their parents/accompanying guardian were also asked about hand washing habits. It was found that 346 children were following hand washing habits. Out of them, 22 harbored parasites. Hygiene status and hand washing habits of the children were significantly associated with parasitic infestation. 370 children were reported with hygienic means of stool disposal. They used toilets for defecation. Out of these children, 22 harbored parasites, 14 children had the habit of defecating in open, and 5 of them had parasites in their samples. The association between stool disposal habits of patients and parasites was statistically significant. 292 children were found to drink usual tap water and 22 of them harbored parasites in their fecal sample. 92 were those who drank water from the hand pump and out of them, 5 children harbored parasites. Peripheral blood smears were prepared for detecting microfilaria larva from the blood samples of participating children but none of the samples showed the presence of microfilaria.

Table 4: Distribution of parasites in participants according to associated factors

Parasites	Eating habits		Hygiene status			Hand washing habits		Stool disposal		Water supply	
	Regular	Other	Bad	Fair	Good	Present	Absent	In-house toilets	Open defecation	Hand pump	Tap water
G. lamblia	8	0	3	5	0	3	5	3	5	0	8
E. histolytica	3	3	2	4	0	6	0	6	0	2	4
H. nana	7	0	0	5	2	7	0	7	0	0	7
B. hominis	2	1	0	3	0	3	0	3	0	2	1
T. hominis	0	1	0	1	0	1	0	1	0	0	1
E. histolytica and *B. hominis*	0	1	0	1	0	1	0	1	0	1	0
E. histolytica, G. lamblia and *B. hominis*	1	0	0	1	0	1	0	1	0	0	1
Test statistics	U=15.00		χ^2=10.47			U=5.00		U=5.00		U=12.50	
P-value	*P*=0.209		*P*=0.005			*P*=0.009		*P*=0.009		*P*=0.112	

G. lamblia: Giardia lamblia, E. histolytica: Entamoeba histolytica, B. hominis: Blastocystis hominis, H. nana: Hymenolepis nana, T. hominis: Trichomonas hominis

DISCUSSION

In the present study conducted among pediatric patients with unexplained eosinophilia, out of 384 participants, 7.03% of the total population was found to be positive for intestinal parasites. None of the peripheral blood smears showed the presence of microfilaria. Most of the positive study participants were from low or middle socio-economic sectors. Very few parents were graduates. 6.5% of children in this category were found to show parasites in their stool samples. Hygiene status, hand washing, and stool disposal habits of patients were found to be significantly associated with the presence of the parasite.

Less number of parasite detection could be due to more awareness of people about health care these days. The most commonly encountered parasite was cysts/trophozoites of *G. lamblia*. In a similar study conducted by Pardo *et al*., among 161 eosinophilic cases, 116 (54.5%) had 1 parasite, 30 (14.1%) had 2, and 15 (7.0%) had >3. Filariae (n = 63, 29.6%) were the most frequently isolated parasite, followed by *schistosomes* (n = 37, 17.4%), hookworms (n = 36, 16.8%), and *Trichuris spp*. (n = 18, 8.4%).[11] This study is in contrast to our study as in our study it was found that protozoa were commonly detected in the participants as compared to helminths. The absence of hookworm in participants of this study can be related to the fact that in rural areas most of the children wear footwear now, being barefooted otherwise could have been a risk factor for few of nematode infections. Blood samples were also taken to detect microfilaria in participants, but none of the filarial cases was detected. This could be

attributed to lesser exposure of children to filarial worms in this locality. The area must not be prone to filarial infections. Modified Kuppuswamy scale uses education and occupation of the head of the family and monthly family income to calculate the socio-economic scale.[12] It was seen that most of the parasite harboring children belong to lower or middle socio-economic backgrounds which could be due to their way of leading life and unavoidance toward risk factors. Our 25 (7.4%) participants belonging to the rural area were found to be positive for parasites. A significant proportion of the population belonging to the rural background is found with no access to safe drinking water or stool disposal. Defecating in the open is a common source for worm infestation. In our study, the ratio between total rural and urban children was found to be 7.1:1 and positive rural and positive urban cases were in the ratio of 12.5:1. In another study conducted by Thakur and Rai in 2016,[13] rural: Urban participants were found to be 13:1. Simple measures like having toilets at each home and health education on safe sanitation and water supply can help particular people from a rural background. It was seen that children with bad personal hygiene were highly associated with parasitic infections (9.61%). A higher prevalence of parasitic infection was seen in children who did not wash their hands after defecation (13.1%). The association between hand washing habits of children and parasitic finding was statistically significant. This shows that although hand washing could have reduced spread of parasites and due to lack of awareness this simple means of sanitary habit is not practiced properly. Children who went for open defecation were found to be more prone to parasitic infections (35.7%). The association between stool disposal habits of patients and parasitic finding was statistically found to be significant. This is the reason due to which government has started many programs which promotes people toward building toilets in every house. Defecation in the open exposes a person to many contaminants/parasites which lead to the diseased condition. Hygienic means of stool disposal/unhygienic means of stool disposal was found to be 1:22 by Thakur and Rai in 2016.[13] This study shows the association between water consumption and parasitic findings to be statistically non-significant. Similarly in another study ratio between underground drinking water supply: River water for drinking was found to be 1.5:1.[13] Among our study participants, 15 were reported of consuming food from outside their home such as market made food and street side fast food. Out of them, 6 (40%) children harbored parasites. This shows that children who consumed food other than regular homemade food were at higher risk. Parasitic infections are also easily transmitted through contaminated food. The association between regular and irregular eating habits of patients was found to be statistically significant. socio-economic scales are also widely used in community surveys and studies to assess the socio-economic status of the family, which is further used as a variable to understand its influence on various diseases and risk factors.

Strengths and Limitations

As this study has been conducted over a short period of time, the major strength is that enrolling patients, collecting data or statistical analysis was completed in a relatively short space of time so that timely appropriate planning interventions can be done. Limitation of the present research work is the cross-sectional nature of the study. A larger confirmatory study is needed to confirm the associations find in this study.

CONCLUSION

Due to lack of education in a rural area, parents are unaware of health care and its benefits, maintenance of hygienic habits and their association with the appearance of diseases. Assessment of parental self-efficacy and child behavior should warrant. Children belonging to lower socio-economic status were highly endured with parasitic infestations. Open defecation and street food also contributed in it. This intriguing finding paves the way for larger studies to confirm this finding and explore the possibility of parasitic infestation as one of the causes of peripheral blood eosinophilia. Possible socio-demographic, environmental and behavioral factors associated with parasitic infestation among pediatric cases with unexplained eosinophilia need to be studied to implement effective control measures so as to provide better health care and health benefits to the children.

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