

Original Research Article

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A Study on the Immune Status of Children with Wheezing and Parasitic Infection

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ABSTRACT

The aim of this study was to identify the parasitic infection and the associated allergic condition by studying the stool samples of the wheezing children and to correlate, the total serum IgE antibody levels in those children. Further to study the type of parasites commonly involved in these rural area. A total of 76 stool and blood samples were collected from the wheezing children attended the allergy clinic at the Pediatric out patient department of Karpaga Vinayaga Institute of Medical Science and Research Centre, during a period of six months. Stool samples of children were examined microscopically for the presence of ova/ cysts/ whole parasite and the body segments using saline and iodine wet mount preparations. Serum samples were tested for total IgE antibodies by Turbidimetric immunoassay. Results were analyzed, among the 76 stool samples examined from the wheezing children, 48 are found to be positive contributing to a prevalence of 63.2 % of intestinal parasitic infection in the study population. The most common intestinal parasite was found to be *Entamoeba histolytica* (33.3%) followed by hook worm (27.1 %) and *Giardia intestinalis* (23 %). Other less commonly detected parasites were cyst of *E. coli* in 5 cases (10.4%), ova of *Ascaris lumbricoides* (6.2%). A high prevalence of intestinal parasites was observed in the wheezing children. Further the children with parasite infection showed a higher level of total IgE in their serum.

Keywords

Wheezing children,
IgE antibody,
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Introduction

Literature survey showed that the wheezing in children in a common respiratory symptoms in allergic condition (Fernando, 2002). Poor hygienic practice may cause parasite infection among children. In rural area the periodical de worming is not done regularly in childhood (Luong, 2004). Parasite infection is one of the triggering factors of wheezing in children

(Kunst *et al.*, 2009). In allergic condition the IgE antibody level will be increased (Manohar *et al.*, 2012). Immunity in secretory regions are maintained by IgA class of antibodies (Harry W. Schroeder *et al.*, 2010). When the IgA level in those regions decreased by any chance, then the microbes may invade the areas such as respiratory and intestinal mucosa (Nicholas J. Mantis *et al.*, 2013). In such conditions the foreign antigens

may induce the allergic response of the host (Norman *et al.*, 1998). Ultimately the IgE antibodies were induced and increased (Thomas, 2001). Therefore it is a fact that IgA and IgE titers are inversely proportional (Michael *et al.*, 2010). In some cases there will be an infection, but there was no induction of antibody against the infection, infers the immunodeficiency of the individual (Andrea Lantz *et al.*, 2001). Hence this study was planned to analyze the immune status of the children showed the signs and symptoms of allergy and to rule out the role of parasitic infection and the associated IgE level in such conditions.

Worldwide about 3.5 billion of population is infected with intestinal parasites, which include mostly children (WHO, 1998). As reported by World Health Organization (WHO) more than 1.5 billion people are infected with contaminated soil (Blouin *et al.*, 2018). These infections are distributed in tropical and subtropical areas, with the majority occurring in developing countries. The warm and moist climate of tropical and subtropical countries provides the ideal environment for the survival of parasite eggs or larvae of these four soil transmitted helminth (STH), roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and hookworm (*Necator americanus*, *Ancylostoma duodenale*) (Brooker *et al.*, 2006). It is mentioned that approximately 270 million children in the preschool-age and nearly 600 million in school-age are living in areas associated with intensive transmission of intestinal parasites (WHO, 2014). There by necessitating treatment and prevention.

In India the prevalence rates greatly varies due to environmental, climatic and socio-economic conditions. Various studies reported on the prevalence of parasitic infection are as follows 49 % in East Godavari of Andhra

Pradesh (Padmaja *et al.*, 2015), 75.28 % in Kashmir (Wani *et al.*, 2007), 51.5 % in Karnataka (Shubha *et al.*, 2011) and 26.88 % in East Delhi (Mahajan *et al.*, 1993). Among the protozoan parasites, *Entamoeba histolytica*, *Giardia intestinalis* and coccidian parasites are common in children. Whereas the most common helminthes detected in children is *Ascaris lumbricoides* and it is the common parasite found in India followed by *Hookworm*, *Hymenolepis nana*, *Tapeworm*, *Trichuris trichiura*, *Enterobius vermicularis*, *Entamoeba histolytica*, *Entamoeba coli* and *Giardia lamblia* (Virk *et al.*, 1994).

Several socio-economic, personal and environmental factors like poverty, illiteracy, lack of safe water for drinking, overcrowding, poor personal hygiene, lack of toilet facilities and several other factors are associated with occurrence of intestinal parasitic infection in children (Padmaja *et al.*, 2014; Mane *et al.*, 2014).

Materials and Methods

Seventy six stool samples were collected from children from the age group of 3 to 13 years, showing signs and symptoms of wheezing who attended the children OPD for treatment at Karpaga Vinayaga Institute of Medical science and Research Centre, Cinnakolambkkam for a period of six months between March 2017 and August 2017.

The study was explained to the parents of the child before collect their personal, medical history and specimens. Further this study was approved by the Institute Ethical Committee. Stool samples were collected in a clean and wide mouth container without contaminating urine. Similarly 2 mL of blood samples were also collected aseptically in a sterile vial from those children and allowed for 20 minutes at room temperature for clot formation. After retraction of the clot the vials are centrifuged

at 2500 RPM for 20 minutes. The clear sera were collected in a vial and labeled. Stool and the serum samples were transported immediately to the Microbiology department. The cross sectional study was carried out in the department of Microbiology.

Identification of parasites in stool

Macroscopic examination

Macroscopic examination of stool includes the observation of odour, consistency, color and presence of blood, mucus or segments of adult worms. Liquid specimens are examined within 30 minutes for trophozoite detection and semisolid specimens within 60 minutes. Preservatives like formalin, polyvinyl alcohol (PVA) were used whenever preservation is needed.

Microscopic examination

Freshly passed stool specimens were collected in clean dry container and are examined immediately. Stool sample were examined microscopically for the presence of ova/ cysts/ whole parasite and the body segments in wet mount preparations with 0.9% saline and Dobell's iodine. Saline mount for the visualization of bile staining property of eggs, live motile larvae or trophozoites and the iodine mount for the in better visualization of finer objects like nuclei. In certain conditions like enterobiasis, peri-anal swab using NIH swab or Scotch cellulose adhesive tape were used. The parasites were identified by their specific morphology of ova/cyst etc.

Estimation of total IgE

The serum samples of the children were tested for the presence of IgE antibodies by Turbidimetric immunoassay using Quantia-IgE kit. A Calibrator curve was constructed using with the given known concentration of

calibrator and its different concentrations prepared by serial dilution with saline. Spectrophotometer was adjusted to read zero with saline at 630nm wave length. As per the procedure of the kit the activation buffer and the Quantia IgE reagents were mixed and incubated in a cuvette for 5 mints at 37°C, and added the calibrator mixed well and incubated. Absorbance values were measured at the end of 10 seconds (A1) and again at the end of 4 minutes (A2). A2-A1 gives the absorbance value (A) of that concentration. The same procedure was repeated with the other serially diluted calibrators and their absorbance values were noted using the Instrument and the values were calculated. A graph was plotted on the absorbance versus the concentration of the calibrators. The same procedure was repeated for each of the serum samples collected from the children and the absorbance values were measured. Making use of the calibrator curve the Total IgE values were calculated for each of the serum sample collected.

Results and Discussion

Characteristics of study population

A total of 76 wheezing children were included in this study, of which 42 were boy children (55.3%) and 34 were girls (44.7%) among the 42 boys 27 were showed positive reports (64.3%) whereas among the 34 girls 21 showed positive (61.8%) for parasites. Majority of children were in the age group of 6-10 years 42 (47.4%) followed by children of age group less than 5 years of age contributed 28 (36.8%). The age group between 11≥13 years only 12 (15.8%). Presence of parasites in various age groups were as follows among the group 3≤5 years 24 (50%), in group 6-10 years 22 (45.8%) and in group 11≥13 years of age only 2 (4.2%) were positive for parasitic infection (Table 1).

Prevalence of intestinal parasitic infections

Among the 76 stool samples examined from the wheezing children, 48 were found to be positive contributing to a prevalence of 63.2 % of intestinal parasitic infection in the study population. The most common intestinal parasite was found to be *Entamoeba histolytica* (33.3%) followed by hook worm (27.1 %) and *Giardia intestinalis* (23 %).

Other less commonly detected parasites were cyst of *E.coli* in 5 cases (10.4%), ova of *Ascaris lumbricoides* (6.2%). Shown in table 2.

It was observed that the cyst of *Entamoeba histolytica* and the eggs of Hookworm are the most commonly present parasites in our study population.

Table.1 Distribution of intestinal parasite in stool samples

Factors	N=76	Parasite	
		+ve =48 (63.2%)	-- ve =28 (36.8%)
Age			
3≤5	28 (36.8%)	24(50.0%)	04(14.3%)
6-10	36 (47.4%)	22(45.8%)	14(50.0%)
11≥13	12 (15.8%)	02(04.2%)	10(35.7%)
Sex			
Boys	42 (55.3%)	27(64.3%)	15(35.7%)
Girls	34 (44.7%)	21(61.8%)	13(38.2%)
Monthly income			
≤5000	13 (17.1%)	11(22.9%)	02(07.1%)
5001-10000	43 (56.6%)	31(64.6%)	12(42.9%)
10001-20000	11 (14.5%)	05(10.4%)	06(21.4%)
>20000	09 (11.8%)	01 (2.1%)	08(28.6%)

Table.2 Prevalence of Intestinal parasites

Sl.no	Parasite	Number of Samples (48)	(%)
1	<i>Entamoeba histolytica</i>	16	33.3%
2	<i>Entamoeba coli</i>	05	10.4%
3	Hookworm egg	13	27.1%
4	<i>Ascaris lambricoides</i>	03	06.2%
5	<i>Giardia intestinalis</i>	11	23.0%

Table.3 Total serum IgE antibody levels in various groups of children

CATEGORY OF CHILDREN	No studied	IgE level (mean±SE IU/mL)	P valve
WITH WHEEZE	76	1194.2±169.4	P <0.001
WITH PARASITE INFECTED	48	1527.0±176	P <0.001
HEALTHY CONTROLS	10	160.4± 57.16	P <0.001

The total serum IgE antibody levels in children

Among the 76 children aged between 3 and 13 years with signs and symptoms of wheeze when tested for the total IgE level using Turbidimetric immunoassay a mean IgE level of 1194.2±169.4 IU/mL was observed. Whereas the total level of Ig E of the parasite infected 48 children showed a mean Ig E level of 1527±176IU/mL. When we tested a control group of 10 healthy children with non wheezing and parasitic infection showed a mean Ig E level of only 160.4±57.16 IU/mL (p<0.001) (Table 3).

Detection of the immune status of the wheezing children infected with parasites were studied by serum IgE and the levels of each of the children were analyzed and were compared and correlated with the reports of the stool sample studies.

Increasing prevalence of wheezing is one of the common chronic disorders of the children. It has been associated with increased exposure to environmental allergens. Literature survey revealed that the bacteria and virus may also cause exacerbation of asthmatic wheezing. The present study was planned to understand the parasitic infection and its role in causing asthmatic wheezing in children. The results showed a lot of parasites infect the gastro intestinal tract in children. Reports suggested the invasive larvae during its migration through the lungs induce a

strong eosinophil rich inflammation in the lungs. Intestinal helminthes infection may also capable of enhancing allergic inflammation (Van den Bigelaar *et al.*, 2000). The study report explained the association of the parasites and its capability of enhancing allergic inflammation. Yet another study it was stated that *A. lumbricoides* was associated with increased risk of Asthma (Leonardi-Bee *et al.*, 2006). In the present study stool samples were collected from the children who were suffered from asthmatic wheeze. Presence of ova of *Ascaris lumbricoides* and some other parasites in the samples of affected children correlate with other studies. Allergy is IgE mediated type I hypersensitivity (Mathias 2011). In normal condition the serum IgE is present in trace amounts. Usually the total IgE level increases with amount of exposure to relevant allergens (Gruchalla *et al.*, 2005). Measurement of serum IgE is useful to determine if the allergic reaction is IgE mediated and its level increases in parasitic infection (Bell, *et al.*, 1996). Various study report indicated a significant fall in IgE concentration after effective therapy. Present study reports conclude that a number of wheezing children showed ova and cysts in their stool samples indicating the parasitic infection. Wheezing children with parasite infection showed a higher level of total IgE in their serum. This base line data may be fine-tuned by further investigations with more number of cases and allergen specific IgE antibody tests.

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