

## The role of worm infestation in allergic rhinitis

Manuel, A.M.<sup>1</sup>, Kuljit, S.<sup>2</sup>, Gopalakrishnan, G.<sup>3</sup>, Suresh, K.G.<sup>4\*</sup> and Balraj, P.<sup>5</sup>

<sup>1,3</sup>ORL Dept, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

<sup>2</sup>Prince Court Medical Centre, 39 Jalan Kia Peng, 50450 Kuala Lumpur, Malaysia

<sup>4</sup>Parasitology Dept, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

<sup>5</sup>Molecular Pathology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia

\*Corresponding author email: suresh@um.edu.my

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**Abstract.** The purpose of this study is to determine the relevance of the hygiene hypothesis; that is to determine if worm infestation has a protective role against the development of allergic rhinitis. A prospective case controlled study was conducted. Specific IgG levels to *Toxocara* were studied in 85 patients confirmed to have allergic rhinitis and were compared to levels in another 85 controls, with no form of allergy. The IgG assay was done using ELISA technique. There was a higher incidence of positive specific IgG to *Toxocara* in the controls as compared to allergic patients. The values were statistically significant [Chi square test (p=0.002)]. This negative association between worm infestation and allergic rhinitis suggests that a previous worm infestation could protect against the development of allergic rhinitis.

### INTRODUCTION

The worldwide incidence of allergic rhinitis varies from 10 - 20% (Bousquet *et al.*, 2001). It poses a significant health problem in terms of patient's quality of life, costs involved and load on health care services. Predisposing factors include genetics, diet, airborne pollution, rising dust mite populations, less ventilation in homes and offices, more sedentary lifestyles and possibly lack of exposure to infections, particularly parasitic infestation (Dykewicz & Fineman, 1998; Bousquet *et al.*, 2001; Cooper *et al.*, 2003)

Riedler *et al.* (2001) noted that a lower prevalence of allergic disorders was recorded in developing countries as compared to industrialized countries in an epidemiological study conducted in 2001. One of the major reasons that might explain the differences in the prevalence of allergies might be the extent of exposure to viruses, bacteria and parasites. Better quality of living standards and easy access to health care system in the urban area have led to the promulgation of hygiene hypothesis to

explain the increase in allergic disorders (Yazdanbakhsh & Boakye, 2005).

The hygiene hypothesis by Strachan (1989) proposed that the stimulation of certain aspects of the immune system by microbes protects from the development of inflammatory diseases like the allergic disorders.

Geohelminth infections have notably reduced the prevalence of allergic disorders in a few studies (Hagel *et al.*, 1993; Cooper *et al.*, 2003). *Toxocara canis* is an important parasitic disease in humans caused by its second stage larvae (L2). It is recognized worldwide especially in developing countries, including Malaysia as a human infection (Hakim *et al.*, 1993; Yamasaki *et al.*, 1998). The infection is acquired through the ingestion of embryonated eggs in sand pits and playgrounds contaminated with dog faeces.

In this study, we evaluate the association between previous geohelminth infection and the prevalence of allergic rhinitis at our centre.

## MATERIALS AND METHODS

This was a prospective case control study involving 170 patients, 85 cases and 85 controls attending the outpatient ENT clinic at our centre over a one year period. Cases were patients newly diagnosed to have allergic rhinitis based on the ARIA guidelines while controls were patients attending the ENT outpatient clinic for non allergic problems which did not affect the immune status. These included deviated nasal septum, vertigo and impacted wax. Exclusion criteria included patients less than 7 years of age, pregnancy and the presence of bronchial asthma and skin allergy. The selected patients were subjected to a questionnaire which included demographic and clinical data.

The incidence of allergic rhinitis among the different races and socioeconomic classes was determined. Socioeconomic class was determined based on the occupation of the patient, with different occupations falling under the following categories: professionals, lesser professionals, semi skilled and unskilled. The incidence of a past worm infestation among the different races and working classes was also determined.

Blood investigations for quantitative measurement of non specific IgE and specific IgG to *Toxocara* using the ELISA technique were carried out for the cases and controls. In the specific *Toxocara* IgG quantitative test, the detection of antibodies against *T. canis* L2 excretory-secretory antigens (TES) was used. Detection of IgG antibodies in human serum using *T. canis* recombinant fusion protein was performed as described previously by Yamasaki *et al.*, 2000). Briefly, each well of microtiter plates (Immunolon II, Dynatech, USA) was coated overnight at 4°C with 100 µl of recombinant *T. canis* antigen at a concentration of 100ng per well in the ELISA plates diluted in coating buffer. The coated plates were washed three times with PBS-0.05%Tween-20 (PBS-T). Blocking buffer (3% skimmed milk) was added into the wells and incubation was carried out for 1 hour at room temperature. Washing was repeated 3 times and the plates were stored in -70°C till used later. 100 µl of patients' serum diluted

1:200 with PBS-T were added into the antigen coated wells in duplicates and incubated for 2 hours at room temperature. Secondary antibody conjugate, peroxidase-labelled goat anti-human alkaline phosphatase conjugate [Kirkegaard and Perry Laboratories (KPL), USA] was diluted 1:100,000 in PBS-T with blocking buffer, and was added into each well, and incubated at room temperature for 2 hours. After three washes, substrate [3,3',5,5'-tetramethylbenzidine (KPL, USA)] was added into the wells and incubated at room temperature in the dark for 4 minutes. Stop reaction, 2.5M sulphuric acid, was added into each well and optical density (OD) measurement was taken at 450 nm using ELISA reader (Dynatech, USA). Positive and negative control sera were added into each test. The mean +4 standard deviation O.D. of 30 healthy volunteers sera was taken as the cut-off value as previously described (Lim *et al.*, 2000; Yamasaki *et al.*, 2000). The sensitivity of the test is >98%, and the specificity is 36%.

Approval to conduct this research was obtained from the Medical Ethics Committee, University Malaya Medical Centre, acting in accordance to the ICH GCP guidelines and the Declaration of Helsinki.

Data collected was tabulated using Microsoft excel programme and analysed using SPSS version 17.

## RESULTS

Of the total allergic rhinitis patients, the highest number were Malays, followed closely by Indians and Chinese (Table 1). Most of the allergic rhinitis patients were students and of the semi skilled working class group (Table 2). The severity of allergic rhinitis was mild in 9 (11%) patients, moderate in 53 (62%) patients and severe in 23 (27%) patients. A previous worm infestation was most common among students and the semi-skilled working group (Table 2). It was also most prevalent among the Malays followed by the Indians and Chinese (Table 1).

Of the 85 patients with allergic rhinitis, 14 (16.5%) were positive for *Toxocara* IgG and 71 (83.5%) tested negative for *Toxocara*

Table 1. Racial distribution among patients with allergic rhinitis and patients/controls with previous worm infestation

	Malay	Chinese	Indian	Others
Controls	25 (29.4%)	31 (36.5%)	25 (29.4%)	4 (4.7%)
Allergic Rhinitis	32 (37.6%)	23 (27.1%)	30 (35.3%)	–
Worm Infestation	20 (43.5%)	12 (26.1%)	14 (30.4%)	–

Table 2. Occupation distribution among patients with allergic rhinitis and patients/controls with previous worm infestation

	Professional	Lesser Professional	Semi skilled	Unskilled	Home maker	Retired	Student	Unknown
Controls	13 (15.3%)	8 (9.4%)	3 (3.5%)	4 (4.7%)	3 (3.5%)	10 (11.8%)	7 (8.2%)	37 (43.5%)
Allergic Rhinitis	4 (4.7%)	9 (10.6%)	14 (16.5%)	7 (8.2%)	5 (5.9%)	4 (4.7%)	15 (17.6%)	27 (31.8%)
Worm infestation	3 (65%)	2 (4.3%)	7 (15.2%)	2 (4.3%)	3 (6.5%)	4 (8.7%)	9 (19.6%)	16 (34.8%)

IgG. In comparison, of the 85 controls, 32 (37.6%) tested positive for *Toxocara* IgG and 53 (62.4%) were negative (Table 3, Figure 1). This difference was statistically significant with a p value of 0.002 using the Pearson Chi-square test, relative risk 3.005 (95% confidence interval).

The IgE level of our negative control was 0, while that of our positive control was >800 IU/ml. IgE levels of the allergic rhinitis patients varied from 60 IU/ml to >800 IU/ml. None of the allergic patients had 0 IgE level. We also compared the IgE levels in allergic rhinitis patients who had a previous worm infestation (as evidenced from an elevated specific *Toxocara* IgG) to allergic rhinitis patients who did not have previous worm infestation (evidenced from negative specific *Toxocara* IgG). 57% of allergic rhinitis patients with a previous worm infestation had IgE levels more than 800 IU/L compared to 65% of allergic rhinitis patients without a previous worm infestation who had IgE levels more than 800 IU/L. However, the difference was statistically insignificant with a p value of 0.5 using the Pearson Chi-square test.

## DISCUSSION

Allergic rhinitis is a type I hypersensitivity reaction mediated by allergen specific IgE antibodies. Allergen deposited on the nasal mucosal surface dissolves and enters the submucosal layer. It is then engulfed by Langerhan cells which process and present the allergen to immunocompetent cells (T & B lymphocytes) (Chung, 2001; Simons *et al.*, 2003). Specific IgE antibody production is encouraged by IL 4, IL 13 and Th2 lymphocytes. IgE binds to receptors on mast cells via its Fc portion. Further exposure of allergen leads to cross linking of two of more IgE molecules which in turn leads to mast cell degranulation and release of

Table 3. Incidence of previous worm infestation among allergic rhinitis patients and controls

	IgG Negative	IgG Positive
Allergic Rhinitis	71 (83.5%)	14 (16.5%)
Controls	53 (62.4%)	32 (37.6%)

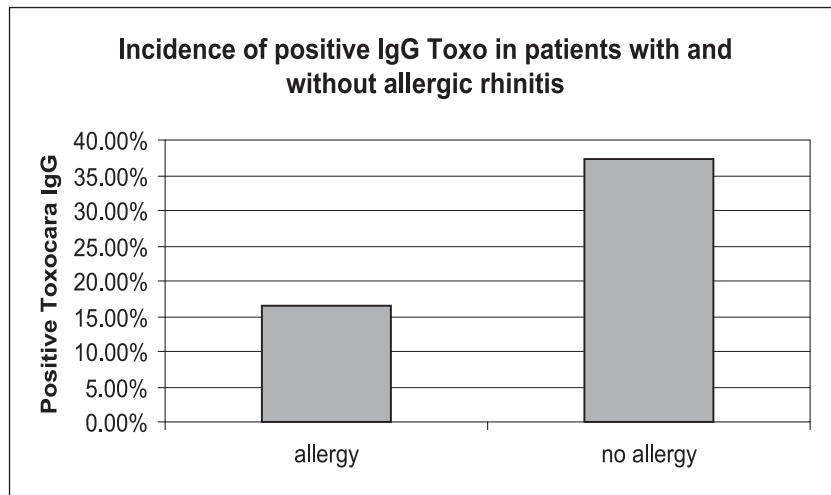


Figure 1. Positive IgG Toxocara in patients with and without allergic rhinitis  
P value = 0.002 using the Pearson Chi-Square Test

mediators of allergic symptoms. A number of suppressors of pro-inflammatory cytokines like IL 10, IL 12 and interferon  $\gamma$  have been found to be decreased in allergic patients and this may reduce their capacity to inhibit IgE synthesis and allergic inflammation (Chung, 2001). The incidence of allergic diseases such as bronchial asthma, allergic rhinitis and eczema has been constantly on the rise over the past few decades (Neil & Goldblatt, 1999). In view of its increasing social and economic impact, there have been innumerable ongoing studies to identify the predisposing factors and more effective treatment modalities. Decrease in worm infestation and increase in allergic diseases appear to have occurred in developed countries (Yazdanbakhsh & Wahyuni, 2005).

The hygiene hypothesis proposes that less frequent exchange of microbes may alter the immune system in such a way that upon encounter of an innocuous antigen such as an aeroallergen, Th 2 responses are readily induced which leads to allergic disease (Strachan, 1989; Yazdanbakhsh & Matricardi, 2004; Yazdanbakhsh & Boayke, 2005). This negative association between worm infestations and allergy has been shown in a study conducted by Cooper *et al.* (2003) in rural Ecuador which showed negative association between geohelminth infections and allergen skin test reactivity and exercise

induced wheeze. Huang *et al.* (2002) also noted a negative association of *Enterobius* infestation with asthma and rhinitis in primary school children in Taipei. Hagel *et al.* (1993) and Nyan *et al.* (2001) noted negative association between allergic disorder with helminth infections.

On the other hand, there have been studies that have shown a positive association or no relationship between allergic rhinitis, bronchial asthma and worm infestation (Sigrid *et al.*, 1998; Kustimur *et al.*, 2006). Sigrid *et al.* (1998) concluded that helminthic infections in East German children were not the cause for a low prevalence of allergies in the former East Germany. Meanwhile Kustimur *et al.* (2006) also found no difference in *Toxocara* seropositivity between asthmatic patients and controls. These inconsistencies might be related to the chronicity of the infestation as acute infestations are associated with increased Th 2 responses while chronic infestations are associated with T cell hyporesponsiveness.

Helminth infections are associated with strong T helper 2 responses, high levels of IL 4, IL 5 and IL 13, increased IgE levels, eosinophilia and mastocytosis. However, chronic helminthic infections are associated with T cell hyporesponsiveness and reduced cytokine production (Yazdanbakhsh

& Boakye, 2005). Down regulatory molecules such as IL 10 and TGF B, which are inflammatory cytokines that are products of regulatory T cells, have been implicated in this immunosuppression. These regulatory T cells have been shown to be associated with lower allergic and autoimmune reactions (Chung, 2001).

In our study, we determined the incidence of previous worm infestation using the presence of IgG antibodies against *T. canis* excretory-secretory antigen. The assay showed cross reactions with other parasitic helminthes commonly found in tropical areas (Yamasaki *et al.*, 1998). However, as the objective of our study was to determine the relationship between worm infestation in general with allergic rhinitis, therefore, this cross reactivity with other parasites did not affect our outcome. Our study suggests the hygiene hypothesis could be true as we found a negative association between a previous worm infestation and allergic rhinitis.

All our patients with allergic rhinitis whether or not they had a previous worm infestation had raised IgE levels; but interestingly, very high levels (>800 IU/ml) were less frequent in allergic rhinitis patients with previous worm infestation. This could mean that though worm infestation may not always prevent allergic rhinitis from developing, it may still reduce the severity of the allergy. However, the difference was statistically insignificant.

The observed differences in the relationship between acute and chronic worm infections with allergic rhinitis warrants further evaluation accompanied with extensive immunological studies to confirm this association. An understanding of any possible contribution to the pathogenesis of allergic rhinitis, true of the hygiene hypothesis provides a potential avenue for prevention.

A previous worm infestation has a potential protective role against the development of allergic rhinitis. This knowledge could be helpful in deriving other methods of preventing or treating allergic rhinitis.

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