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Clinical and laboratorial parameters analysis related to atopy in children from urban and rural zones

Análise de parâmetros clínicos e laboratoriais relacionados à atopia em crianças da zona urbana e rural

Análisis de parámetros clínicos y laboratoriales relacionados a la atopía en niños de la zona urbana y rural

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The objective this study was analyze the expression of allergy (dermatitis, rhinitis and/or asthma) e relate it to the incidence of parasites' infections. A clinical study was made on children from 2 to 15 years old inhabiting urban or rural zone. The evaluation was made by the ISAAC adapted quiz and laboratorial exams - counting of IgG4, total IgE and specific to Derp 1 2, Per a7, mite's tropomyosin and from Ascaris, eosinophil counting and parasitologyc feces' exam. The prevalence of parasite infections was reduced on the studied population, but was elevated in the population resident at rural zone. It observed correlation between the total concentration of IgE and the relative eosinophil counting. The occurrence of allergies or the precedence of the people wasn't related to laboratorial alterations, nor to reactivity to parasite and environmental antigens, due to the fact that the studied city with low social, environmental and climatic discrepancies between rural and urban zones.
Descriptors: Hipersensitivity; Immunoglobulin E; Helminths.

O objetivo deste estudo foi analisar a expressão de alergias (dermatite, rinite e ou asma) e relacionar com a incidência de parasitoses. Foi feito um estudo clínico em crianças de 2 a 15 anos residentes na zona urbana ou rural. A avaliação se fez pelo questionário ISAAC adaptado e exames laboratoriais - dosagem de IgG4, IgE total e específico para Derp 1 2, Per a7, tropomiosina de ácaro e de Ascaris, contagem de eosinófilos e parasitológico de fezes. A prevalência de parasitoses foi reduzida na população estudada, mas elevada na população proveniente de zona rural. Observou-se correlação entre a concentração de IgE total e contagem relativa de eosinófilos. A ocorrência de alergias ou a procedência dos indivíduos não esteve relacionada com alterações laboratoriais, e nem com reatividade a antígenos parasitários e ambientais, por se tratar de uma interiorana, com poucas discrepâncias sociais, ambientais e climáticas entre zona rural e urbana.
Descritores: Hipersensibilidade; Imunoglobulina E; Helmintos.

El objetivo de este estudio fue analizar la expresión de alergias (dermatitis, rinitis y/o asma) y relacionarla con la incidencia de parasitosis. Fue hecho un estudio clínico en niños de 2 a 15 años residentes en la zona urbana o rural. La evaluación se hizo por el cuestionario ISAAC adaptado y exámenes laboratoriales - dosificación de IgG4, IgE total y específico para Derp 1 2, Per a7, tropomiosina de ácaro y de Ascaris, conteo de eosinófilos y parasitológico de heces. La prevalencia de parasitosis fue reducida en la población estudiada, pero elevada en la población de la zona rural. Se observó una correlación entre la concentración de IgE total y conteo relativo de eosinófilos. La incidencia de alergias o la procedencia de los individuos no estuvo relacionada con alteraciones laboratoriales, ni con reactividad a antígenos parasitarios y ambientales, por tratarse de una ciudad del interior, con pocas discrepancias sociales, ambientales y climáticas entre zona rural y urbana.
Descriptores: Hipersensibilidad; Inmunoglobulina E; Helmintos.

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INTRODUCTION

An increase in the prevalence of alergic diseases, especially asthma, rhinitis and atopic eczema has been recently noted, affecting about 30% of the world's population. Allergic rhinitis represents a global public health problem that affects at least 10 to 25% of the general population, and its prevalence is also increasing. Between 5 and 20% of children from around the world are affected by atopic dermatitis and 60% of these continue presenting the disease after puberty[1].

Asthma is one of the most common chronic diseases of childhood. More than 200 million cases are estimated in the world, constituting a real epidemia[2]. In addition, approximately 80% of children with atopic dermatitis are at risk of developing respiratory allergy. Patients with atopic dermatitis are more sensitive to environmental allergen exposures, and when exposed to high concentrations of inhaled allergens, severe eczema[3] can be triggered.

Inadequate sanitation, commonly observed in rural communities, is associated with a higher prevalence of intestinal parasites. However, there are frequent reports of ascariasis in urban areas, similar or even superior to the rural areas that surround cities in the third world[4]. In addition, there are important connections between inadequate sanitation and exposure to environmental allergens, especially those derived from cockroaches and dust mites, as well as in triggering food allergies[5-8].

Epidemiological studies have shown an inverse association between the prevalence of asthma and atopy and the exposure to infections (viruses, bacteria and parasites) and bacterial products (endotoxin) [9]. A "hygiene hypothesis" was developed from these evidences. It states that infections inhibit the development of atopy: populations with high prevalence of intestinal parasitic infections will have a greater production of IgE, and would be protected from allergic diseases[10]. Several studies over the years aimed at demonstrating a clearer connection between intestinal parasitic infections and the occurrence of asthma and other atopies, although some comments are contrary to this theory [11].

Recent findings indicate that resistance to ascariasis in atopic individuals could occur through the production of IgE and the expression of Th2 cytokines to antigens of the parasite. These individuals would have a greater histamine release by basophils than children without allergies[12]. Regarding this relationship between helminths and allergens, the existence of similar antigens in parasites and mites was demonstrated, for example, in the cysteine protease of *Ancylostoma duodenale* and the Der p1 of the genus Dermatophagoides. That may be related to exacerbation of allergies in patients infected, or even greater resistance to infection by helminths[13].

The controversy of results observed in several studies demonstrates the high complexity of the interaction between allergy and helminthiasis, probably influenced by factors restricted to the populations and environmental factors. In this context, the objective of this study was to analyze the expression of allergies (dermatitis, rhinitis or asthma) and establish a connection between them and the incidence of parasitic diseases.

METHOD

The population between 2 and 15 years of age was constituted by approximately 470 individuals [14], and 335 of these were studied, indicating that the cases in this study are representative enough.

A peripheral blood collection was carried out through venipuncture for CBC and dosage of antibodies. Stool samples were collected for parasitological examination. A questionnaire was applied – the International Study of Asthma and Alergies in Childhood (ISAAC), which was adapted to the investigation of asthma, allergic rhinitis and atopic dermatitis cases. This reseearch was approved by the Research Ethics Committee of the Federal University in the Triângulo Mineiro (UFTM), Protocol n° 909.

The blood test was performed at the laboratory of clinical pathology in the General Hospital of UFTM. The differential count of eosinophils was conducted through a smear test stained by the Giemsa method.

Regarding the parasitology of feces, 3 samples were collected from each child. They were obtained on alternate days, without prior use of laxatives, in a vial containing MIF (merthiolate, iodine and formaldehyde). The techniques of direct examination and a modified variety of Hoffman's test, were described briefly: *Direct Examination:* two to three drops of saline were dropped 0.85% onto a microscopy slide. Small portions of various points of the stool sample were added. They were spread and were later smear stained with lugol's iodine. The sample was observed in an optical microscope with zooms of 10x and 40x. *Modified Hoffman's Test:* approximately 5 g to 10 g of feces were placed in a disposable bottle, together with approximately 10 ml of water, and it was then thoroughly crushed. Another 10 ml of water was added, and the suspension was filtered through a 15 ml tube with a lid, by using a surgical gauze folded in four parts. This suspension was left to stand for 2 hours. It was centrifuged to 220g, for 10 minutes, after what the supernatant was discarded. The sediment was shook in an electrical shaker, and one drop of the result was placed in a microscope slide to which two drops of lugol were added. The sample was observed in an optical microscope with zooms of 10x and 40x.

The serum levels of lgG4 and the lgE total were determined by the ELISA method, using pairs of monoclonal antibodies that are commercially available (eBioscience, San Diego, CA, USA). 96 well dish cell culture plates were briefly used, and made sensitive through the use of specific monoclonal antibodies in order to capture lgG4 or lgE, according to the manufacturer (eBioscience, USA). After incubation, the dishes were cleaned, and blocked with a phosphate buffered saline (PBS), containing 2% of bovine albumin (Sigma, St. Louis, MO, USA). After incubation, the dishes were cleaned, and the samples added, diluted to a 1:2 ratio in PBS/BSA 2%. After incubation the dishes were cleaned and incubated with detection antibodies together with HRP (eBioscience, USA). After incubation the dishes were cleaned and a TM substrate (eBioscience, USA) was added. The results were obtained through the difference between the obtained absorbances of 450 and 570 nm (Abs 450- Abs 570), and was expressed in absorbance.

A chimeric ELISA was used to quantify the presence of IgE specific to Derp1-2 antigens (INDOOR, USA). The ELISA was conducted according to the methodology previously described by Araujo *et al*., 2000[15], and its result was expressed in absorbance or in UI/mL, the concentration obtained directly from the control curve.

Nominal data was described according to its absolute and percent occurrence. To analyse the numerical data, the Mann-Whitney U test and the independent Student's "t" test, to compare the variables between 2 groups; Spearman's rho was used to evaluate the connection between the quantitative data. The statistical analyses were conducted through the use of the program *StatView Abacus Concepts* , version 4.57 (Statsoft, USA). The significance level considered to the tests was of 5% ($p<0.05$).

RESULTS

335 children from the city of Veríssimo - MG were evaluated through spontaneous demand, outpatient caring and active search at schools. From them, 167 were female (49%) and 168 were male (51%). In addition, 249 (74%) lived in an urban area and 86 (26%) in a rural area. According to data from the last population census, Veríssimo had 3,486 residents, being that 58.4% lived in an urban area and 41.6% in a rural zone[14].

It was observed that 117 children did not present any atopy (34.9%), and 218 children (65.1%) presented some kind of atopy (asthma, rhinitis or eczema), being that 89 children had asthma (26.6%), 162 had rhinitis

(48.4%) and 97 eczema (29%). Regarding the connection between the diseases, asthma and rhinitis have presented themselves simultaneously in 57 children (17%), asthma and eczema 37 (11%) and rhinitis and eczema in 65 (14.9%). When compared to the global atopy occurrence, a meaningful difference could not be observed (Table 1).

Table 1. Atopy Occurrence according to the procedence of individuals. Veríssimo, 2009.

Atopy	Urban Zone	Rural Zone	Total
Present	163 (65.7%)	53 (61.6%)	216
Absent	85 (34.3%)	33 (38.4%)	118
Total	248 (100.0%)	86 (100.0%)	

No statistically meaninful difference. Statistical analysis were conducted through Fisher's exact test, with a result of p=0.514.

Regarding the parasitological stool test, only 30 samples (8.95%) were positive. From the positive samples, 5 (1.5%) children from urban zones presented positive tests for *Giardia sp*; 24 (7.2%) presented positive tests for *Entamoeba*, from these, 17 were from urban zones and 7 were from rural zones. Only one child (0.3%) from an urban zone was positive for *Ascaris lumbricoides*. Generally, there has been a meaningfully higher rate of parasitological stool samples in children who come from rural zones (Table 2).

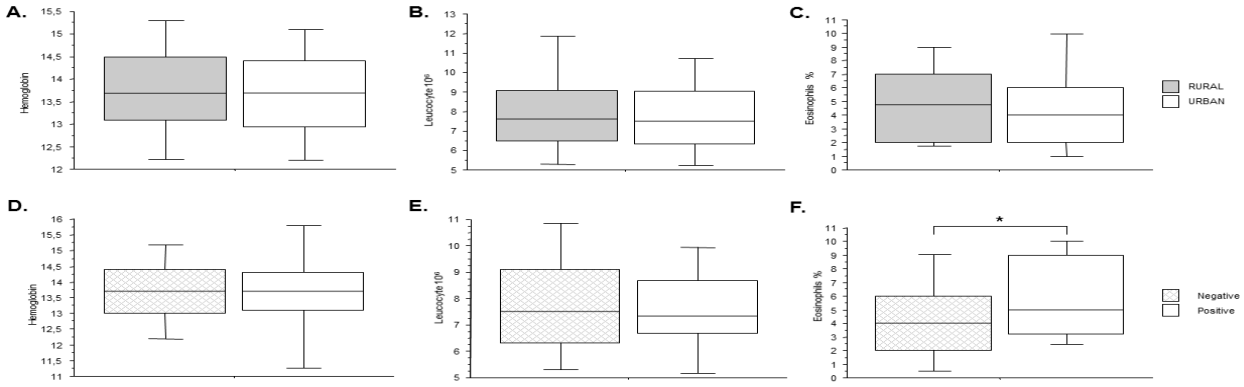
Table 2. Positive results in parasitological stool test according to the origin of the individuals. Veríssimo, 2009.

PARASITOLOGICAL	Urban Zone	Rural Zone	Total
Positive	15 (6.0%)	14 (16.3%)	29
Negative	233 (94.0%)	72 (83.7%)	305
Total	248 (100.0%)	86 (100.0%)	

Statistical analysis through Fisher's exact test, p=0.0006.

No meaningful statistical differences were found in the hemoglobin concentration, in the hematocrit, or in the RBC (red blood cells) count, when comparing individuals originated from urban or rural zones. The results were the same for individuals whose parasitological stool test was negative or positive (Figures 1A and 1D). The total leukocyte count, such as the differential leukogram was not statistically meaningful when it comes to the origin of the individuals or the result of the parasitological stool test (Image 1B and 1E). The count of eosinophil's, whose increase could be related to atopy or to the existence of helminthiases, has not presented meaningful differences, due to the origin of the individuals, rural or urban (Image1C), but they were meaningfully elevated in patients with a positive parasitological stool text (Image 1F). Even though, the percent of eosinophils was not different from one allergic condition-asthma, rhinitis or eczema — to the other (data not shown).

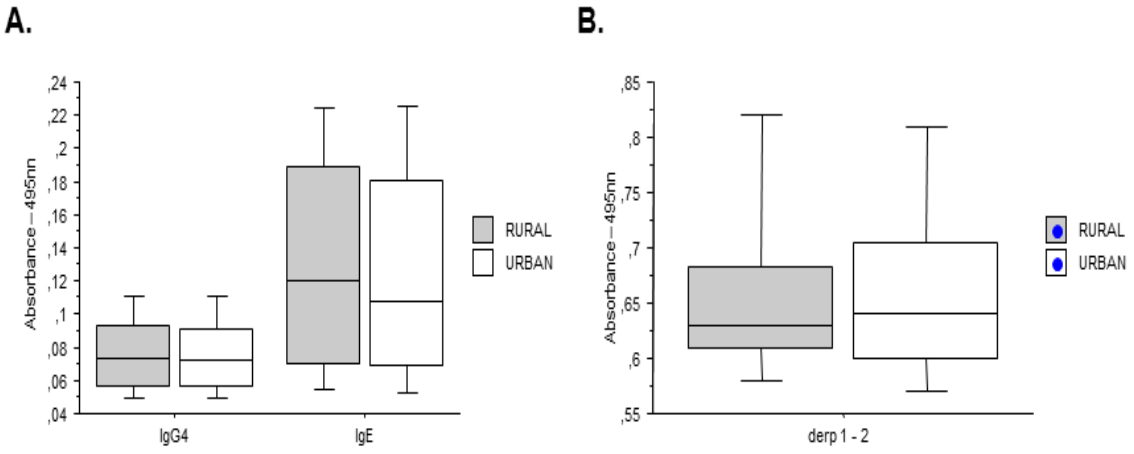
Image 1. Blood cell parameters according to the origin of the individuals and the parasitological stool test. Veríssimo, 2009.



Hemoglobin levels, total leukocytes and eosinophil percent according to individuals originated from urban or rural (A-C) settings and positive or negative results in the parasitological stool test (D-F). The horizontal line presents the median, the bars the percents 25-75% and the vertical lines the percents 10-90%. Statistical analysis made through the Mann-Whitney test.

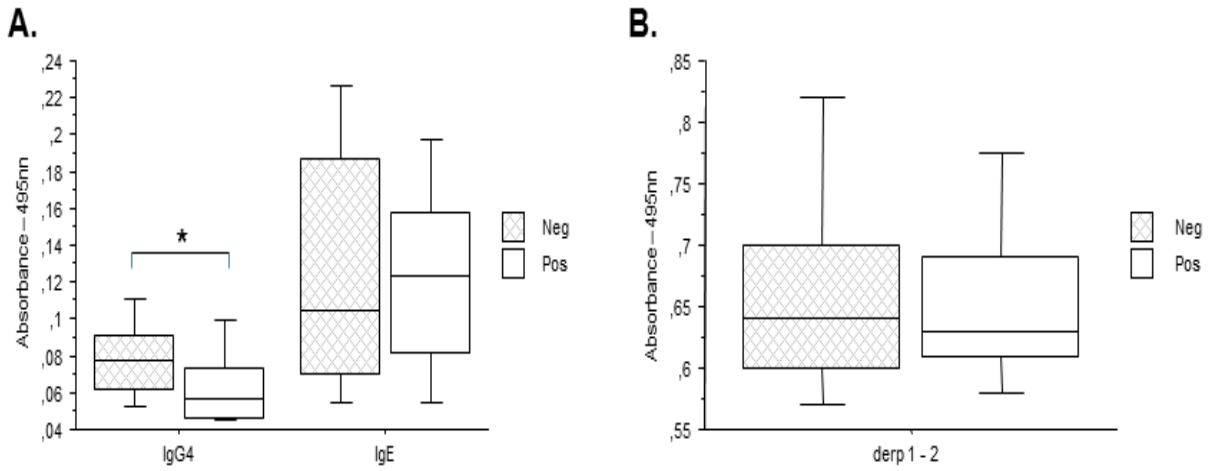
There was no difference among the patients regarding lgG4 or plasmatic lgE, neither regarding their urban or rural origins (Image 2A) nor specifically to lgE anti-derp 1-2 (Image 2B). Regarding rural or urban origins, no meaningful difference could be found in IgE or IgG4 levels (data not shown). Regarding IgG4, a statistically meaningful increase can be noted in individuals whose parasitological stool test was negative, when compared to those with a positive result (Image 3A). Among these individuals, no difference in the total IgE or IgE anti-derp 1-2 levels could be noticed (Image 3B).

Image 2. IgG4 levels, total IgE and IgE anti-derp 1-2, according to the individuals' origin. Veríssimo, 2009.



IgG4, IgE (A) and IgE anti-derp 1-2 (B) among individuals who came from urban or rural zones. The horizontal line presents the median, the bars the percents 25-75% and the vertical lines the percents 10-90%. Statistical analysis made through the Mann-Whitney test (B-D).

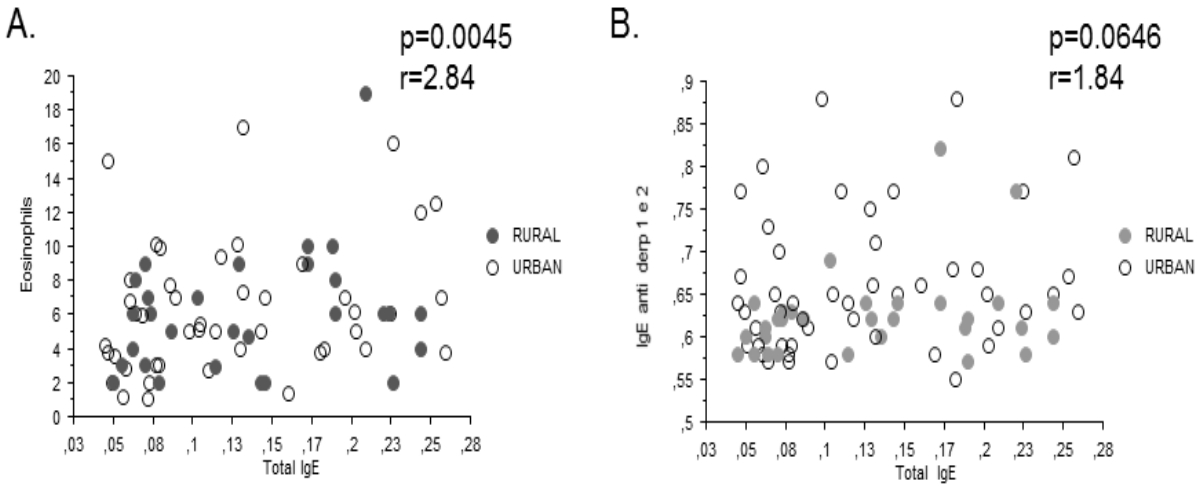
Image 3. IgG4 levels, total IgE and IgE anti-derp 1-2, according the parasitological stool test. Veríssimo, 2009.



Count of IgG4, IgE (A) and IgE anti-derp 1-2 (B) among individuals whose test results were positive or negative in the parasitological stool test. The horizontal line presents the median, the bars the percents 25-75% and the vertical lines the percents 10-90%. Statistical analysis made through the Mann-Whitney test.

The data shows a meaningful and positive connection between relative eosinophil counts and plasmatic levels of total IgE (Image 4A). On the other hand, the plasmatic concentration of total IgE and the reactivity of antigens Der p 1-2 did not present meaningful connections (Image 4B).

Image 4. Connection between total IgE, eosinophils and anti-derp 1 and 2 IgE. Veríssimo, 2009



Connectino between total IgE and eosinophils (A) and anti-Derp 1-2 (B). Statistical analysis conducted through the use of Spearman's correlation.

DISCUSSION

The conection between allergies and helminthiasis is controversial. Cooper *et al.*, while evaluating Ecuadoran patients, reported that a helminthic infection had negative influences in the atopy[16] and Flohr *et al*. had the same results in patients in Vietnam[17]. However, another study did not present any influences from the helminth infection in atopies or even showed it to be a factor that worsen allergies[18]. In Brazil, Nascimento-Silva *et al*., have shown that there is a high helminthiasis prevalence (57%), associated to a high asthma prevalence (60%)[19].

In this study, the prevalence of asthma and rhinitis was similar to that described by Solé[20], who evaluated 20 Brazilian cities. However, the occurrences of eczema was bigger in this study. On the other hand, a study conducted in Belo Horizonte found that approximately 75% of children had rhinitis[21]. In the study presented here, rhinitis was also the most common allergic manifestation, but its prevalence was not as large (48.4%).

No difference was found in the red blood cell count indexes regarding the origin of the participants or the occurrence for atopy, nor was any found among the hosts of intestinal parasites. However, since there were not enough positive results for the parasitological stool test, it is not possible to conduct a broader analysis. Since the blood cell count test parameters are within normal limits, all children were in good general health. They were also in good basic sanitation a condition, which contributes for the control of these parasite infections, especially when associated to the treatments against this type of infection that are offered by the public health system.

No differences were observed in the incidence of atopy, asthma, rhinitis and eczema, when it comes to the gender or the origin of the individuals. This result may be a reflection of the similarity between urban and rural settings in the town evaluated. The individuals originated from rural environments presented a greater prevalence of intestinal parasites. This result is in accordance with studies in which the results for helminthiasis were more positive in rural regions, or in those where basic sanitation was lacking[18].

The determination of anti-*ascaris* IgE is capable of detecting the infection even in individuals with a negative parasitological test, and it can be negatively associated with the presence of eggs in the stool sample[23]. Medeiros *et al.* suggest that, among patients who present allergic conditions, the determination of anti-*A*scaris IgE is more sensitive than the parasitological stool test in the finding of helminthic infections[24]. The levels of total IgE in the patients who presented some atopy symptoms, be it asthma, rhinitis or eczema, did not present total IgE levels which were statistically different from those of non-atopic individuals. Previous articles have conflicting results, sometimes showing a greater concentration of IgE in atopic individuals[25], sometimes not showing any difference between atopic and non-atopic individuals[26]. Still, epidemiological data indicate that the inhibition of the reactions to aeroallergens in individuals infected by geohelminths is not associated to IgEs serum levels [18].

It has been suggested that intestinal parasite infections, especially when caused by helminthes, influences the total levels of IgE[27], even though a study conducted with a larger group of individuals did not confirm this influence, whether it be in the urban or rural zone[25]. The concentration of IgE have shown itself to be related to the precent of eosinophil's. Similarly, Satwani et al.[26] observed a strong correlation between eosinophil and the total levels of IgE, as well as to the presence of allergy. However, the connection between eosinophil and IgE levels has not been meaningful in some systemic allergic diseases.

In addition to IgE, IgG4 antibodies were previously correlated both with anti-parasite immunity and allergic reactions[28,29]. This study has shown that children with a negative parasitological stool test were shown to have a greater concentration of these antibodies. Even

considering that the incidence of parasite infections was higher among children who came from rural settings, there was no difference in the IgG4 due to the origin of the participants. Previous studies have pointed out that there is an inversion between protection and pathogeneses in these classes of antibodies when it comes to allergies and parasite infections, especially helminthiases, and that effector anti-parasite mechanisms are more dependent on IgE than on IgG4[30-32].

Several aspects such as the age of the patient when he or she suffered a parasite infection for the first time, parasite load, socioeconomic conditions, style of life, and exposition to environmental allergens can have important roles in the connection between parasite infections and allergic diseases. Events that happened when the child was about 2 or 3 years old can be very important in the development of allergic diseases[33]. The choice of 2 years of age as a minimum age was based on the indications in the literature, which suggest that the immune system, specially the innately responsive immune system, reach full maturation when the child is around 24 months old[34].

Previous studies have shown that acari allergens, the *D. pteronyssinus* and *B. tropicalis*, are the most prevalent in the dust of most tropical regions[33], and that there is a connection between sensitivity to these dust mite and asthma[35]. The reduction in the exposure to this allergen increased significantly the symptoms and exacerbations of the disease[36]. In the population evaluated, the results tended to a connection between the levels of total IgE and anti-Derp1, which could suggest that the exposure to this type of allergen has a higher impact in the serum IgE levels than possible cases of intestinal parasite. A correlation was also noticed between the levels of total IgE and the increase in the number of eosinophil's. Studies have suggested that, in childhood, increases in the concentration of IgE serum levels are mainly caused by allergic phenomena, and not by the presence of parasite infections[34].

CONCLUSION

The incidence of phenomena such as hypersensitivity and the origin of individuals were not connected to the changes in laboratory parameters, especially when there is reactivity to parasitic antigens and allergens. However, it was shown that the antibodies that work against acari antigens can be important causes of the IgE total levels, and the reactivity to acari antigens is accompanied by an increase in the reactivity to antigens Per a7.

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CONTRIBUTIONS

Jussara Silva Lima redacted the research project, oriented and supervised the collection of the exams, conducted immunological exams, data analysis and the writing of the article. **Carlos Alberto Mota Araújo** took part in the collection of exams and in the analysis of the parasite stool test analysis. **Luisa Karla de Paula Arruda** oriented the conduction of immunological exams and their analysis in her laboratory at USP Ribeirão Preto. **Valéria Cardoso Alves Cunali** performed a critical reading and helped in the writing of the article. **Virmondes Rodrigues Junior** oriented and supervised the project, the data colecction, and the final version of the article. **Vandui da Silva dos Santos** took part in the data analysis and in the writing of the article.

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