Asthma in Tanta, Egypt: Serologic Analysis of Total and Specific IgE Antibody Levels and their Relationship to Parasite Infection

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Abstract. The relationship between asthma, IgE and parasitic infection was compared in 68 randomly selected patients with asthma and 37 nonasthmatic controls living in Tanta, Egypt. Sera were assayed for total IgE and for IgE antibodies to inhaled allergens (mite, cat, cockroach, rye grass, ragweed and 3 fungi) and to parasite antigens (Schistosoma mansoni and Brugia malayi). Parasite infection was determined by microscopic examination of stool specimens. Total IgE levels were significantly higher in patients with asthma (geometric mean 909 IU/ml), than in controls (geometric mean 145 IU/ml, p < 0.001). The high IgE levels correlated with parasite infection and the presence of IgE antibodies to S. mansoni antigens, which were also elevated compared to controls. The prevalence of allergen-specific IgE antibodies among Egyptian asthmatics was low by comparison with Western asthmatics, but nonetheless higher than among Egyptian controls. A radioallergosorbent test (RAST) values of > 40 U/ml to any allergen was found in 19/68 (28%) sera from the asthma group, as compared to only 1/37 (3%) sera from controls (p < 0.001). The highest RAST values were to dust mite (Dermatophagoides pteronyssinus and D. farinae) allergens, followed by rye grass and ragweed allergens. The results suggest that in this area of Egypt, several factors may influence the development of asthma, including nonspecific activation of IgE and/or inflammatory mechanisms by helminth parasites and sensitisation to environmental allergens.

Introduction

In the West, asthma is commonly associated with elevated serum IgE levels [1]. Previous studies have also demonstrated a higher prevalence of IgE antibodies to common inhaled allergens among patients with asthma than among nonallergic controls [2-5]. Other evidence for a causative role for allergen exposure in asthma has come from allergen avoidance studies, from analysis of the prevalence of allergen-specific IgE antibodies in patients admitted to hospital emergency rooms with asthma, and, most recently, from a prospective study of the development of asthma in children [6-10]. Recent evidence also suggests that atopy (i.e., the predisposition to develop IgE antibodies) is inherited as an autosomal character, tentatively localized to the long arm of chromosome 11 [11].

The general conclusion from these studies is that individuals with the genetic predisposition to make IgE antibody responses will become sensitized upon exposure to a certain level of allergen, and that continued exposure increases the risk of developing allergic diseases, particularly asthma.

In populations in which parasitic (helminth) infection is endemic, total serum IgE levels can be extremely high (1,000-50,000 IU/ml) [12-15]. Helminth infection stimulates the production of specific anti-parasitic IgE as well as a large excess of IgE that is not specific for parasite antigens but for environmental allergens [16, 17]. Asthma surveys conducted in areas where parasitic infection is endemic have led to 2 conflicting hypotheses [reviewed in ref. 18]: that parasitically induced IgE, by blocking mast cells, may prevent asthma [19-22], and, conversely, that parasitic infection through a hypersensitivity response to the allergenic components of the parasite may predispose to the development of asthma [23-25]. Thus, the relationship between parasitic infection and asthma is unclear and measurements of total serum IgE in individuals living in the affected areas give little guidance about the probability of having allergic diseases.
In the present study, the relationship between asthma IgE antibody levels and parasitic infection in Tanta, Egypt, was investigated.

Materials and Methods

Subjects
Sixty-eight patients referred from the Clinic of Chest Diseases, Tanta University Hospital, Tanta, Egypt, with a diagnosis of bronchial asthma based on history and clinical examination, were enrolled in the study. All patients showed symptoms of intermittent breathlessness and/or wheeze more than 20% improvement in 1-second forced expiratory volume (FEV1). Values, within 30 min of inhalation of 2 puffs of salbutamol (β2-receptor stimulator), FEV1 was measured using Jones Pulmonary III Waterless Spirometer supplied with a “Datamatic III” computer. Adjustment of readings were done according to age, sex and height of each patient as well as room temperature. Thirty-seven volunteers (students, hospital staff, or faculty) with no history of asthma were enrolled in the study as a control group.

Both the patients with asthma and control subjects answered a brief questionnaire and provided a serum sample. A fecal specimen from each subject was examined microscopically for detection of intestinal parasites [26].

Immunofluorescence for Total and Specific IgE Antibodies
Total serum IgE was measured by using a two-site monoclonal antibody-based enzyme immunoassay [8, 27]. Briefly, polystyrene microtiter plates (Dynatech Immunon II) were coated overnight with 2 monoclonal anti-IgE antibodies (CIA/E/712 and CIA/E/443) kindly provided by Dr. Andrew Saxon, University of California, Los Angeles, Calif., USA. Serum samples were added at dilutions of 1:100, 1:500, and bound IgE was detected using biotin-labelled affinity purified goat anti-human IgE (Kirkegaard and Perry Laboratories, Gaithersburg, Md., USA). Results were interpreted from a computer-generated reference serum, that contained 100 IU IgE/mL. This reference had been subsampled against the US National Institute of Health IgE standard (A-699-000-500) containing 800 IU IgE/mL.

IgE antibodies to isolated allergens were measured by a quantitative radioligand-receptor test (RAST) using allergen extracts coated to oxynorm bronchial-activated cellulose discs as described previously [8]. The allergens used were Dermatophagoides pteronyssinus, D. farinae, cat epithelium, cockroach extract (German, American and Oriental mixed), short ragweed pollen, ryegrass pollen and fungal extracts (Trichophyton mentagrophytes Asperillus fumigatus and Alternaria alternata). Extracts were obtained from Hollister-Stier, Spokane, Wash., USA.

IgE antibody to Schistosoma mansoni antigen and Brugia malayi filarial antigens were measured by RAST using adult worm extracts kindly provided by Dr. Eric A. Ottesen, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease, Bethesda, Md., USA. [26].

Values for IgE antibodies to both isolated allergens and parasite antigens, were quantitated relative to a standard D. farinae RAST curve. This curve was established using D. farinae discs and serial 2-fold dilutions of a mix allergic serum pool (UVA 8701) containing 1,000 U/ml of IgE antibodies to D. farinae. The units were standardized relative to the International Reference Serum Pool established at the National Institute of Biological Standards and Control in London (NIHSC Code No. 92/525), which contains 1,800 IU of IgE antibodies to D. farinae (1 U of IgE antibody in the RAST is approximately equivalent to 0.3 ng of IgE antibody) [8, 26, 30]. For each antigen, the assay background was established using 4 sera from nonatopic, skin-test-negative donors. In addition, at least 1 positive serum containing a known quantity of allergen-specific IgE was tested in each assay.

Statistical Analysis
Total IgE and specific IgE antibody levels in patients with or without asthma were compared using Yates’ corrected z2 test. Student’s t test or Fisher’s exact test. Geometric means (GM) were calculated for the ages and total IgE levels of patients and controls.

Results

Study Area and Patient Population
Egypt (lat. 21°35'–33°25'S; 25°–36°E) is a dry subtropical area and the climate is characterized by a 2-season year: a relatively cool winter from November to March (mean temperature 14°C) and a hot summer from April to October (mean temperature 30°C). Tanta, the capital of the El Gharbia governorate, lies in the middle of the Nile Delta 56 miles north of Cairo (fig. 1). According to the 1987 census, the population of Tanta was 887,000 persons, of whom 379,000 were living in Tanta city, while 557,000 were living in the surrounding villages. The average population den-
Table 1. Study populations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Asthmatics (n = 68)</th>
<th>Controls (n = 37)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years; geometric mean</td>
<td>20.92</td>
<td>20.05</td>
<td>NS</td>
</tr>
<tr>
<td>Range</td>
<td>18–67</td>
<td>20–56</td>
<td></td>
</tr>
<tr>
<td>Sex, males/females</td>
<td>32/36</td>
<td>18/19</td>
<td>NS</td>
</tr>
<tr>
<td>Residence, urban/rural</td>
<td>14/54</td>
<td>30/7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Personal history of other allergic diseases</td>
<td>11 (16.2)</td>
<td>1 (2.7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Family history of asthma</td>
<td>19 (27.9)</td>
<td>1 (2.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Current smokers</td>
<td>12 (17.6)</td>
<td>6 (16.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>8 (11.8)</td>
<td>0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Positive stool exam for parasites</td>
<td>46 (67.6)</td>
<td>5 (13.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figures in parentheses are percentages.

* Significance of observed differences between asthmatics and controls was evaluated by Yates' corrected x² test except for age (Student t test), other allergic diseases and previous smoking (Fisher's exact test). NS = Not significant (p > 0.05).

* These include: for asthmatics: nasal allergy (6 cases), skin allergy (4 cases) and aspirin hypersensitivity (1 case); for controls: skin allergy (1 case).

* First-degree relatives only.

* These include: for asthmatics: S. mansoni (26 cases), A. lumbricoides (n = 19), E. histolytica (n = 13), E. vermicularis (n = 11), and Ancylostoma duodenale (n = 3); for controls: S. mansoni (n = 3), E. histolytica (n = 2), A. duodenale (n = 1), and Hymenolepis nana (n = 1). Combined infection was common.

Table 2. Distribution of total serum IgE levels

<table>
<thead>
<tr>
<th>Total serum IgE IU/ml</th>
<th>Asthmatics</th>
<th>Controls</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n %</td>
<td>n %</td>
<td></td>
</tr>
<tr>
<td>&lt; 300</td>
<td>15</td>
<td>22.06</td>
<td>26</td>
</tr>
<tr>
<td>300–600</td>
<td>11</td>
<td>16.18</td>
<td>4</td>
</tr>
<tr>
<td>600–900</td>
<td>8</td>
<td>11.76</td>
<td>4</td>
</tr>
<tr>
<td>900–1,200</td>
<td>4</td>
<td>5.88</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 1,200</td>
<td>30</td>
<td>44.12</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>100.00</td>
<td>37</td>
</tr>
<tr>
<td>GM</td>
<td>908</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>95% c.l.</td>
<td>645–1,288</td>
<td>89–234</td>
<td></td>
</tr>
<tr>
<td>p*</td>
<td>&lt;0.001</td>
<td></td>
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</tr>
</tbody>
</table>

c.I. = Confidence limits.

* t test performed on logged data.

It was 1,450 persons/km², one of the highest densities in the world.

Sixty-eight adult patients with asthma and 37 control subjects were enrolled in the study between December 1989 and February 1990. There were no significant differences between the groups in terms of age, sex or current smoking habits (Table 1). A significantly greater proportion of asthma patients reported either a personal or family history of allergic disease, as compared to controls (p < 0.05). There were also highly significant differences between areas of residence and prevalence of parasitic infection between the two groups (Table 1). Most patients with asthma (80%) lived in rural areas, and 66% of these patients had detectable ova or cysts of intestinal parasites in stool specimens (principally S. mansoni, A. lumbricoides, Ennforma hisotylnga and Enterobius vermicularis). Of the patients with asthma and parasitic infection, 44/46 (96%) lived in rural areas. Similarly, although most of the controls lived in Tanta City, 45 patients with parasitic infection were rural residents.

Total IgE Levels and their Relationship of Parasitic Infection

Total serum IgE levels among patients with asthma were very high (GM 908, range 45–2,280 IU/ml). Half of these patients had IgE levels of > 900 IU/ml compared to only 2/37 control subjects (Table 2). The majority of the controls had total IgE levels of > 300 IU/ml (GM 145, range 14–2,968 IU/ml), and the differences between the IgE levels of patients with asthma compared to controls were highly significant.
IgE Antibodies in Egyptian Asthmatics

(p < 0.001, table 2). The results were further analyzed according to parasite infection (fig. 2). Among patients with asthma, all but 1 of those with >900 IU IgE/ml had a positive parasite examination, whereas only 2/15 with IgE levels <300 IU/ml were infected. Thus, the asthma patients could be divided into those with concomitant parasite infection and high IgE levels (>900) and those without apparent infection, with lower serum IgE levels (<300). These groups accounted for 72% of all patients. The remaining patients had moderately elevated IgE and approximately 60% had a positive parasite examination. The IgE levels of the Egyptian asthmatics and controls were compared with those of ‘Western’ (US) asthmatics and control subjects. These patients (114 asthmatics and 114 controls) had been enrolled in a study of Emergency Room Asthma in Wilmington, Del., USA [30]. The patients with asthma had presented with acute airway obstruction, whereas the controls presented with any diagnosis other than asthma. The results showed that the mean IgE levels of Egyptian asthma patients were approximately 5-fold higher than those of US patients (GM 160 IU/ml; fig. 2). Moreover, the mean IgE levels of the US patients were not significantly different from those of the Egyptian controls. The high IgE levels of Egyptian controls may in part reflect previous parasitic infection.

Specific IgE Antibodies

There was a low prevalence of IgE antibodies to inhaled allergens in both Egyptian patients with asthma and controls (table 3). Among patients with asthma, approximately 20% had a moderate to strongly positive RAST (>40 U/ml) to mite allergens, and approximately 10% to either ryegrass or ragweed pollens. The highest IgE antibody levels were to mite allergens (up to 4,600 U/ml). The detection of IgE antibodies to ragweed was rather surprising, and it was not clear whether this represented ‘imported’ short ragweed (Artemisia artemisiifolia) or cross-reactivity with another weed pollen. The prevalence of IgE antibodies to cat and cockroach allergens was very low, with only 3 patients having a RAST >40 U/ml to either of these allergens. In an attempt to identify other potential allergens, IgE antibodies to selected fungal allergens were measured. Two patients with asthma had IgE antibody to Trichophyton tonsurans; 1 patient had IgE antibody to A. alternata; and IgE antibody to A. fumigatus was not detected (data not shown). In analysing the RAST data, values of >40 U/ml were regarded as significant levels of IgE antibody and values of 10–40 U/ml as equivocal, taking into account the high total IgE level which could give false-positive results over the lower range. The prevalence of IgE antibodies to mite and pollen allergens was significantly higher among patients with asthma than among control subjects and only 1 of the controls had a RAST >40 U/ml (table 3).

Levels of specific IgE antibodies to parasite antigens were also measured. The results were in keeping with the observed differences in parasite infection between the study populations and showed that a significantly higher proportion (78%) of patients with asthma had circulating IgE antibody to adult S. mansoni antigen, as compared to controls (16.2%, p < 0.001). Neither asthma patients nor controls had significant IgE antibody to B. malayi, a filarial parasite which is not found in the area.
Table 3. Prevalence of IgE antibody responses to inhaled allergens and parasitic antigens in asthmatics and controls

<table>
<thead>
<tr>
<th>IgE ab</th>
<th>RAST U/ml</th>
<th>D. farinae</th>
<th>D. pteronyssinus</th>
<th>Cockroach</th>
<th>Cat</th>
<th>Ragweed</th>
<th>Ryegrass</th>
<th>S. mansoni</th>
<th>B. malayi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n %</td>
<td>n %</td>
<td>n %</td>
<td>n %</td>
<td>n %</td>
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<td>n %</td>
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<tr>
<td>Asthmatics (n = 68)</td>
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<tr>
<td>&gt; 200</td>
<td>3 4.4 3 4.4 0 0.0 0 0.0 1 1.5 2 2.9 4 5.9 1 1.5</td>
<td></td>
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<tr>
<td>40-200</td>
<td>4 5.9 11 16.2 2 2.9 1 1.5 7 10.3 5 7.4 24 35.3 0 0.0</td>
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<td></td>
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<tr>
<td>10-40</td>
<td>5 7.4 3 4.4 4 5.9 3 4.4 8 11.8 8 11.8 25 36.8 0 0.8</td>
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<tr>
<td>Total</td>
<td>12 17.7 17 25 6 8.8 4 5.9 16 23.6 15 22.1 53 78 1 1.5</td>
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<td>Controls (n = 37)</td>
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<tr>
<td>&gt; 200</td>
<td>0 0.0 0 0.0 0 0.0 0 0.0 0 0.0 0 0.0 0 0.0 1 2.7</td>
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<tr>
<td>40-200</td>
<td>0 0.0 0 0.0 0 0.0 0 0.0 0 0.0 0.0 0 0.0 1 2.7 5 13.5 0 0.0</td>
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<tr>
<td>10-40</td>
<td>1 2.7 1 2.7 1 2.7 0 0.0 2 5.4 1 2.7 1 2.7 0 0.0</td>
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<tr>
<td>Total</td>
<td>1 2.7 1 2.7 1 2.7 0 0.0 2 5.4 2 5.4 6 16.2 1 2.7</td>
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<tr>
<td>Range</td>
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</table>

p* <0.05 <0.01 NS NS <0.01 <0.05 <0.001 NS

* Fischer’s exact test. NS = Not significant (p > 0.05).

Discussion

Although it is well known that parasitic infection is common in this area of Egypt, the relationship between allergy, asthma and parasitic disease has not been widely studied. A recent population survey suggested that the prevalence of adult asthma in Egypt is 3.3% [31], i.e., similar to estimates in several western countries. The results show that in this group of randomly selected patients with asthma living in Tanta, total serum IgE levels were very high, but allergen-specific IgE antibodies were low by comparison with asthma patients living in the West (where the prevalence of allergen-specific IgE antibodies among asthmatics can be up 80%). Broadly, patients from Tanta could be distinguished into 3 groups: (1) the majority, with high serum IgE levels and parasitic infection, but no evidence of allergen-specific IgE antibodies; (2) an additional approximately 30% of patients with high IgE levels, parasite infection and IgE antibodies (RAST > 40 U/ml) to inhaled allergens, and (3) patients with low total IgE (< 300 IU/ml), only 3 of whom had a positive RAST to any of the allergens or parasites tested.

The prevalence of allergy among asthma patients in Tanta was significantly higher than among controls and, in fact, the prevalence among controls was remarkably low (<3%). The low prevalence of allergen-specific IgE antibodies in the asthma patients could be explained by limited environmental allergen exposure or an effect of parasitic infection. Dust mites and plant pollens appeared to be the predominant allergens. The overall prevalence may be slightly underestimated because a limited panel of 9 aeroallergens was used for RAST testing. Some patients may have IgE antibodies to other allergens, such as mosquitos (Prosopis spp.) pollens, or insects (chironomids), which have been reported to cause allergic symptoms in other parts of the Middle East (Kuwait and Sudan) [32, 33]. Given the dry, arid conditions (<1" of rainfall/year), it is perhaps surprising that the highest RAST values were against dust mite allergens. However, Frankland and El-Hefny [34] originally identified D. culmorum (now D. farinae) in house dust samples from Cairo and 6 mite species (D. farinae, D. pteronyssinus, Acheles gracilis, Bemisia tabaci, Tyrophagus putrescentiae and Cheyletus sp.) have subsequently been found in homes in Tanta [35]. Our re-
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Results suggest that there must be significant mite allergen exposure in houses in Tanta and that mites are one of the most important aeroallergens in the area. Houses in Tanta are usually uncarpeted, but the use of cotton bedding is common and bedding appears to be the major site of mite infestation. During the summer months (May-September), the mean indoor temperature and relative humidity (22°C and 60% RH) provides optimal conditions for mite growth [35]. Allergy diagnosis in Egypt is hampered by the lack of availability of suitable extracts for either skin testing or in vitro IgE antibody assays. The present results suggest that inhaled allergens could be responsible for asthma symptoms in some patients and that better allergy diagnosis and further investigation of the relationship between allergen exposure, sensitization, and clinical symptoms would be worthwhile.

The most significant disease associations observed in the present study were between asthma and parasitic infection, particularly among patients living in rural areas. A previous study by Khed [36] investigating bronchial asthma among school children in a rural village near Tanta, suggested that parasitic infection was not primarily responsible for the development of asthma, but that asthma was more severe among children with concomitant parasitic infection. Clinical observations in the area further suggested that patients with asthma and parasitic infection experience greater exacerbations of symptoms and usually improve when treated with antihelmintic drugs. There is evidence that parasites can affect asthma symptoms by causing physical damage to the lung or through immunological effects, including recruitment of eosinophils and other inflammatory cells, T-cell activation, mediator release, and nonspecific activation of IgE-producing B cells [38]. These effects would be expected to exacerbate inflammatory reactions occurring in the lung. The findings of asthma and allergen-specific IgE antibodies in parasitized individuals argues against the view that parasite infection 'protects' against the development of asthma [19-22].

In conclusion, the results suggest that in the Tanta area of Egypt, asthma is a multifactorial condition, associated with elevated total IgE levels, parasitic infection and IgE antibody responses to inhaled allergens. The results also raise the possibility that parasitic-induced activation of IgE production and cellular inflammatory responses could influence the development of asthma in this area.

Acknowledgements

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References


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