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Revisiting the Hygiene Theory; Hepatitis A and Tuberculosis Versus Atopy

Yavuz Özer[1](ID), Haluk Çokuğraş[2](ID)

[1]Department of Pediatrics, Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Istanbul, Turkey
[2]Division of Pediatric Allergy and Immunology, Department of Pediatrics, Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Istanbul, Turkey,

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Abstract

Objective: According to the hygiene hypothesis an inverse association between allergic sensitization and exposure to infections and has been reported. In this study, we investigated the relationship between atopy and tuberculosis (TB) and hepatitis A virus (HAV) infections in children.
Methods: A cross-sectional study was performed and included 39 healthy children who were followed up with TB, 40 healthy children who were with HAV seropositive, and 30 healthy children who were seronegative for HAV and tuberculin skin test (TST) response as negative. Serological tests for HAV (anti-HAV immunoglobulin G), skin prick test (SPT) investigations for the detection of atopy, and TST were carried out.
Results: The study included 39 (16 males, 23 females) with TB, 40 (16 males, 24 females) with HAV seropositive, and 30 (10 males, 20 females) healthy controls. There was no statistically significant difference between the groups in terms of age and gender ($p>0.05$). The SPT positivity was 28.2% (n=11) in the TB group, 15% (n=6) in the HAV group, and 30% (n=9) in the control group. There was no statistically significant difference between the groups in terms of SPT positivity ($p=0.148$). There was no statistically significant difference between the groups in terms of total serum IgE level ($p=0.776$).
Conclusion: Our study does not support the hypothesis that HAV and TB suppress the development of atopy. We think that encountering infections during the immune maturation period is a condition that is protective in the development of atopy due to multifactorial reasons

Keyword: Hygiene hypothesis, asthma, atopy, hepatitis A, tuberculosis

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Address for correspondence/reprints: **Telephone number:** +90 (507) 036 22 69

Yavuz Özer **E-mail:** yavuzcp@yahoo.com

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INTRODUCTION

It is seen that there has been a significant increase in the prevalence of allergic diseases all over the world in the last century (1,2). According to the hygiene hypothesis, the interaction of the natural environment and microorganisms with the immune system plays an important role in the regulation of the immune system and the development of allergic diseases. Changes in diet and the use of antibiotics affect the content and diversity of the human microbiome. Also, lifestyle and environmental changes cause changes in exposed microorganisms (1). The duration of exposure to these microorganisms and exposure in the early period of life is effective in the development of atopy. In addition, mode of delivery, breastfeeding, early human contact, number of siblings, and farm life play a role in the development of atopic diseases (3).

David Strachan first suggested in 1989 that "unhygienic contact" and infections in early life can prevent allergic diseases (4). Advances in T helper lymphocytes type 1 (Th1) / Th2 theoretically support the hygiene hypothesis (5). Subsequently, the hypotheses of "old friends" and "disappearing microbes" have also been associated with an increase in autoimmunity, cancer, and allergic diseases (6,7).

Infections can have different effects on a developing immune system. It is thought that certain infections may have a protective effect on allergic diseases depending on the characteristics of the infectious agent or the immune response of the host (3). The protective effect of infectious diseases against atopic diseases tuberculosis (TB) and measles, which are known to be potent inducers of Th1 response, have been studied. In addition, it is thought that some infections such as hepatitis A virus (HAV), Helicobacter pylori, and Toxoplasma gondii, which are thought to be indicators of hygiene deficiency, may be effective in preventing the development of allergic diseases (8–10). The absence of infections considered potentially protective may be associated with having a small number of siblings, excessive 'hygiene,' and the use of antibiotics and vaccines (10). Conflicting results have been reported in studies investigating the relationship between infections and atopy to date (11–16).

In this study, we investigated the effects of HAV and TB on atopy, evaluating the skin prick test (SPT) results and serum IgE levels of HAV seropositive and TB cases in comparison with a healthy control group without HAV and TB.

METHODS

Study Methods and Population

A cross-sectional study was performed in the tertiary pediatric allergy and clinical immunology center. The sample selection was performed using the simple random sampling method from the pool of cases that visited the outpatient clinic within one year. This study included 39 healthy children who were followed up with TB, 40 healthy children who were with HAV seropositive, and 30 healthy children who were seronegative for HAV and TST response as negative. Patients who had chronic pulmonary disease, malignancy, and primary immune deficiency were excluded based on clinical and laboratory screening (17). After the demographic and clinical characteristics of the cases were recorded from medical records, tuberculin skin test (TST) and SPT were performed and total serum IgE values were investigated. The results were compared with age and gender-matched controls. The diagnosis of TB was made based on clinical and radiological findings as well as microbiological identification. Hepatitis A was diagnosed with total HAV IgG antibody positivity in cases that were not administered the HAV vaccine. The diagnosis of TB was based on clinical and radiological findings, along with microbiological identification, and all patients had a history of former TB infection. Hepatitis A was diagnosed based on total HAV IgG antibody positivity in individuals who had not received the HAV vaccine, and none of the Hepatitis A patients had been vaccinated.

The Clinical Research Ethical Committee of Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty approved the study (Project Number: 14647). We state that the parents have given their written informed consent to be involved in the study, in accordance with the Declaration of Helsinki.

Tuberculin skin test

The tuberculin test values performed on the flexor side of the forearm of all cases with 5 tuberculin units were evaluated and recorded after 48-72 hours. Cases with a tuberculin test induration diameter of $\geqslant$ 10 mm were considered tuberculin reactivity. In addition, cases with a tuberculin reaction $\geqslant$ of 10 mm were investigated radiologically by family screening for possible tuberculosis disease.

Total serum IgE level

Those with a total serum IgE value above 100 IU/mL were considered significant in terms of atopy. Total serum IgE was tested nephelometrically using the BN2 nephelometer device (Siemens, Munich, Germany).

Skin-prick tests

All cases underwent the same SPT panel. Twenty-five most common allergens (Allergopharma, Reinbek, Germany) were used for the SPT. Allergens used for the test are grasses (velvet grass, fruit grass, crazy grass,

forest grass, meadow timothy grass tail, tea leaf), trees I mix (early flowering: alder, elm, hazelnut, poplar, willow), trees 2 mix (late flowering: birch, beech, oak, plane), weed mix (cart-track, weed, Xanthium strumarium), grasses/cereals (grass pollen mix with, wheat, barley, oat, rye), molds I-II (*Aspergillus fumigatus, Alternaria alternata, Cladosporium herbarum*), *Dermatophagoides farinae, Dermatophagoides pteronyssinus,* Mugwort, Birch, Nettle, Alder, Hazel, Rye, Engl plantain, Dog epithelia, Cat epithelia, Sheep's Wool, Peanut, Strawberry, Cacao, Cow's milk, Banana, and Tomato. Histamine (1.7 mg/mL) was used for positive control and isotonic (saline) for negative control. An SPT was performed on the forearm flexor skin of the cases. The reactivity was measured after 15 minutes. After the negative control value was removed in the SPT, the skin tests of the cases that showed reactivity of 3 millimeters or greater to at least one allergen were accepted as positive and evaluated as atopic.

Serological Tests

For serological evaluation, 5 milliliters of serum samples were taken from the cases and stored at -20 C until serological analysis tests were performed. Total HAV G antibody in serum samples was investigated by the ELISA method (DIA.PRO, Milano, Italy). Cases who were positive for HAV IgG antibodies and had not been vaccinated before were considered to have encountered HAV infection.

Statistical analysis

All the analyses performed were done by using the IBM SPSS 21.0 program (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk tests were used to check whether the continuous variables were normally distributed. The categorical variables were presented as numbers (percentages). The continuous variables were given as median values with the 25th and 75th percentiles (25p and 75p, respectively). The categorical variables were compared with the Chi-square test or Fisher's exact test. The continuous variables without normal distribution were compared with the Kruskal Wallis test (for three groups). The p-value <0.05 is considered significant.

RESULTS

The study included 39 cases of TB, 40 cases of HAV, and 30 healthy controls. There was no statistically significant difference between the groups in terms of age and gender, and it was found that they were comparable with each other ($p>0.05$).

The median age of the TB group was 9.8 (7.5-12.9) years. In the TB group, 82.5% of the cases were followed up for pulmonary TB and 17.5% for extrapulmonary TB. Ten percent of extrapulmonary TB cases were followed up due to lymphadenitis, 2.5% for peritonitis, 2.5% for skin involvement, and 5% for bone involvement. Microbiologically, the rate of identification of bacilli was 25%. There was a

history of atopic disease in 6 cases (15.4%) in the TB group. Four cases had a history of asthma and 2 cases had allergic rhinitis. There was no case with a history of HAV.

The median age of the HAV group was 11.0 (8.5-12.3) years. Five percent of the cases in the HAV group had a history of HAV. Only 1 case (2.5%) had a history of allergic rhinitis. There was no case with a history of TB.

Table 1. Demographic features and atopic status of the study groups.

	TB group (n=39)	**HAV seropositive group (n=40)**	**Control group (n=30)**	**p-value**
Age (year), median (q1-q3)	9.8 (7.5-12.9)	11.0 (8.5-12.3)	11.0 (9.0-12.2)	0.851[a]
Male/Female, n (%)	16/23 (41/59)	16/24 (40/60)	10/20 (33.3/66.7)	0.786[b]
Age at diagnosis (year), median (q1-q3)	8.0 (6.0-12.0)	-	-	-
History of atopy, n (%)	6 (15.4)	1 (2.5)	4 (13.3)	0.117[b]
History of TB n, (%)	39 (100)	-	-	-
History of HAV, n (%)	-	2 (5)	-	-
Family history of atopy, n (%)	1 (2.6)	2 (5)	2 (6.7)	0.713[b]
Family history of TB, n (%)	19 (48.7)	2 (5)	-	<0.001[b]*
Number of siblings, median (q1-q3)	1 (0-2)	1 (1-2)	1 (1-2)	0.447[a]
Pet keeping, n (%)	-	2 (5)	1 (3.3)	0.387[c]
Parental smoking, n (%)	29 (74.4)	22 (55)	17 (56.7)	0.155[b]
Tuberculin reactivity (mm), median (q1-q3)	16 (8-21)	0 (0-10)	0 (0-6)	<0.001[a]*
SPT positivity, n (%)	11 (28.2)	6 (15)	9 (30)	0.148[b]
Total serum IgE level (IU/mL)	24.0 (14.3-60.0)	26.0 (13.8-75)	33.5 (14.3-72.5)	0.776[a]
Anti-HAV IgG seropositivity, n (%)	18 (46.2)	40 (100)	-	<0.001[b]*

Abbreviations: TB: tuberculosis, HAV: hepatitis A virus, SPT: skin prick test, IgE: immunoglobulin E, IgG: immunoglobulin G, a: Kruskal Wallis tests, b: Chi-square test, c: Fisher's exact test, *: p<0.001

Table 1 legends: There was no inverse relationship between the hepatitis A virus (HAV), the tuberculosis (TB) group, and atopy.

The median age of the control group was 11.0 (9.0-12.2) years. There was a history of atopic disease in 4 cases (13.3%) in the control group. Three cases had a history of asthma and 1 case had allergic rhinitis. There was no case with a history of HAV or TB.

There was no statistically significant difference between the groups in terms of number of siblings, pet keeping, and parental smoking (p>0.05).

The median tuberculin reactivity was significantly higher in the TB group compared to other groups (p<0.001). The median tuberculin reactivity was not statistically significant between HAV and control groups. (p=0.498).

No allergic symptoms were observed in any of the cases while performing SPT. The SPT positivity was 28.2% (n=11) in the TB group, 15% (n=6) in the HAV group, and 30% (n=9) in the control group. There was no statistically

significant difference between the groups in terms of SPT positivity (p=0.148).

There was no statistically significant difference between the groups in terms of total serum IgE level (p=0.776). Table 1 summarises the demographic features of the study group.

DISCUSSION

In the present study, it was found that there was no reverse relationship between HAV seropositive and former TB patients and atopy (positivity of SPT) in children. SPT positivity was 28.2% in the TB group, 15% in the HAV group, and 30% in the control group.

Our results showing that there is no relationship between HAV seropositivity, TB history, and atopy contradict the hygiene hypothesis. These different results may be due to the small sample size, cross-sectional design, and use of self-reported data to qualify the results in our study. We postulate that certain unexplored variables in our study, including the method of delivery, breastfeeding, and genetic predisposition, may significantly contribute to the modulation of the infant's microbiota and subsequent implications for the development of allergic diseases and asthma (3,18).

In our study, SPT positivity was 15% in the HAV group. In the study of Kocabaş et al (19), SPT positivity was found to be 4.8% in the HAV seropositive group, while the SPT positivity in the healthy control group was found 32.2%. While the protective effect of HAV seropositivity has been demonstrated in studies made in developed countries (13,16), could not be demonstrated in studies made in underdeveloped societies (14,15). According to the Hispanic Community Health Study/Latino Study (HCHS/SOL) study failed to show associations between asthma and H. pylori or HAV seropositivity among large and diverse Hispanic/Latin adult populations (20). One possible explanation is that due to the different socioeconomic levels and vaccination programs, exposure to infection occurs at different ages (19). It has been suggested that the programming of memory T cells occurs in early childhood (11,15). Exposure to the infection at different ages causes different immunomodulation responses (21). Despite HAV IgG positivity, the absence of HAV history is associated with the fact that HAV infection in early childhood is often completely asymptomatic (22).

In our study, SPT positivity was 28.2% in the TB group. Anlar et al. reported that SPT positivity in active and inactive TB cases was found to be 9.5% and 8.3%, respectively. In the same study, SPT positivity was 31% in the healthy control group. They suggested SPT reactions can be suppressed in cases with TB(11). On the contrary, there are studies showing that it has no preventive effect on the development of atopy (12,23). The reason for the different results between the studies could be due to the different study designs, applied

BCG strains and doses, evaluation of test results, environmental factors, and genetic immune responses (24,25). In studies claiming that TB is protective against atopic diseases, it is suggested that the stimulation of Treg cells and anti-inflammatory pathways inhibit allergic inflammation (26). This may be due to the Th2 response that develops after a strong Th1 response during active TB, as a result of changes in an immune balance due to an adjuvant effect of M. tuberculosis or anti-TB drugs (11).

The prevalence of atopy has been shown to decrease as the variety of microorganisms exposed increases (9). It is thought that the effects of different infections on the risk of developing atopy may be different. C difficile, which usually colonizes the gut after antibiotic therapy, is more common in people living in a more "sterile" environment. This invasion disrupts the mucosal barrier and facilitates the entry of antigens (13).

Growing in a hygienic environment with less microbial exposure is thought to increase the atopy response by altering the Th1/Th2 balance (9). The low prevalence of atopy in type 1 diabetes cases with Th1 dominant response and high atopy prevalence in chronic hepatitis B virus carriers with insufficient Th1 response support the Th1/Th2 model (27). It has been suggested that higher serum IL-10 levels as a result of previous infections may be protective against atopic diseases (24). The fact that helminthic infections are associated with a lower prevalence of atopy, despite their Th2 response, contradicts the hygiene hypothesis (6). It has been suggested that the relationship of these infections with atopy is due to their anti-inflammatory response via TGF-beta and IL-10 (25).

The increased prevalence of allergies in people migrating from regions with low allergy prevalence to regions with high allergy prevalence is thought to be due to changes in environmental factors (28). The increase in asthma prevalence cannot be explained by the hygiene hypothesis alone (29). In addition, genetic background is another important factor that determines the atopy phenotype (21). The high rate of family history of atopy indicates the importance of genetic predisposition in atopic individuals (9). Gene polymorphisms that demonstrate the heterogeneity of the asthma phenotype and have been shown to cause increased susceptibility to asthma have been identified (9). T-cell immunoglobulin and mucin domains-containing protein 1 (TIM1) gene polymorphism have been shown to affect the severity of the disease in HAV seropositive individuals and protect them from atopic diseases (30). Possible interactions of environmental and genetic factors have been blamed for the increased prevalence of allergic diseases in developed countries (9).

Current approaches provide symptomatic relief but do not reduce the prevalence of atopic

diseases. Therefore, there is a need to develop preventive strategies for atopic diseases (3). Additional factors associated with microbiome interactions, such as exposure to allergens, and environmental pollution, may contribute (31). It is thought that recovery of human microbiota may help to reduce allergic disease risks (1). However, the evidence regarding the potential benefits of the administration of probiotics, prebiotics, or bacterial lysates is not yet sufficient (32). Wherein the objective, the protection of flora consisting of non-pathogenic microorganisms or is recovered.

In our study, objective measurements, including skin prick tests, serum IgE levels, and tuberculin skin tests, were used to assess atopy. The limitations of our study were its single-center design, cross-sectional nature, and inclusion of small sample sizes. We believe that generalizing this result would not be accurate due to the small sample sizes. Since this study is a cross-sectional study, there is no temporal relationship between atopy and exposure to infections. Therefore, it is not feasible to establish definitive conclusions regarding the cause-and-effect relationship between exposure to infection and atopy. A larger sample size, longitudinal, prospective, and multicenter studies, is necessary to confirm the relationship between atopic diseases and past infections.

CONCLUSION

In our study, which used objective methods, an inverse relationship was not found between exposure to infection and atopy. We think that the assumption that exposure to infections such as TB and HAV, which are frequently encountered in poor hygienic and low socioeconomic conditions during the immune maturation period, protects from atopy, is a condition that occurs due to multifactorial (genetic, developmental, and environmental) reasons.

Ethics Committee Approval: The study was approved by the local ethics committee of Istanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty (Project number: 14647).

We state that the parents have given their written informed consent to be involved in the study, in accordance with the Declaration of Helsinki.

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