

Original article

Association of hepatitis A virus infection with allergic sensitization in a population with high prevalence of hepatitis A virus exposure

Background: An inverse association between allergic sensitization and markers of exposure to food-borne and orofecal infections (particularly hepatitis A virus, HAV) has been reported. The prevalence of HAV exposure and allergic sensitization vary widely in different areas, and vary along with age within a given area.

Aim: To investigate the association between HAV exposure and allergic sensitization in adults from a mostly rural area of Spain.

Methods: An age-stratified random sample of 720 subjects was drawn from the population older than 18 years of A-Estrada, Spain. From 697 eligible subjects, 469 (67.2%, median age 54 years, range: 18–92) participated in the study.

Positive skin prick tests to a panel of aeroallergens defined allergic sensitization. Positive serum HAV antibodies (assayed in 465 subjects) defined HAV exposure.

Results: The prevalence of HAV exposure was 83.6% (95% CI: 80.7–86.5). The prevalence of allergic sensitization was lower in subjects with HAV exposure than in patients without it (25.0% vs 40.0%, OR 0.44, 95% CI: 0.25–0.77, $P = 0.004$), but this association became substantially altered after adjusting for age, which was closely linked to both allergic sensitization and HAV exposure (adjusted OR 1.15, 95% CI: 0.60–2.19, $P = 0.66$).

Conclusions: In a population with high prevalence of HAV exposure, no significant association between HAV exposure and allergic sensitization is observed after controlling for the confounding effect of age.

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The 'hygiene hypothesis' proposes that allergic disease could be prevented by infection in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally (1, 2). These infections in early life are postulated to induce a polarized T helper (Th1) response able to prevent the Th2-biased balance in individuals prone to allergic diseases (2–6), although additional mechanisms cannot be excluded (4). According to the hygiene hypothesis, declining exposure to infections in socioeconomically developed countries may be responsible for the rising trend of allergy and asthma (2–4). Supporting this hypothesis, serologic evidence of exposure to certain infections (mainly food-borne and orofecal infections as surrogate markers of poor hygiene), is associated with a lower probability of having allergic sensitization and allergic diseases in adult subjects (2–4, 7–11).

The most consistent evidence of the inverse association between infection and allergy is provided from studies of hepatitis A virus (HAV) exposure (2, 7–11). The HAV, an orofecally transmitted picornavirus, is a common cause of hepatitis worldwide (12). The HAV is endemic in

developing countries, where most residents are exposed in childhood (12). In contrast, developed countries demonstrate falling rates of HAV exposure along with improvements in hygiene and sanitation (12, 13). Reinforcing this relationship is that a progressive fall in the prevalence of HAV exposure in developed countries has been paralleled by a progressive increase in the prevalence of allergic diseases (4). The HAV may be not only a surrogate marker of poor hygiene, but may be also an immune modulator (6, 14). Recent studies suggest that HAV might provide protection from allergic sensitization by binding to the TIM1 receptor, directly limiting the development of Th2 cells (6, 14).

The prevalences of HAV exposure (12) and allergic sensitization (15) vary widely in different countries. Moreover, the prevalence of both HAV exposure and allergic sensitization varies with age (10, 11). The present study was aimed at evaluating the association between HAV exposure and allergic sensitization in adults of a wide range of ages from a predominantly rural area of Spain.

Material and methods

Study population and design

This cross-sectional study formed part of the A-Estrada Allergy Study. The detailed description of setting and sampling has been reported elsewhere (16, 17). The study was performed in the municipality of A-Estrada, in northwest Spain. The municipality has an area of 282 km² and had an adult population (18 years and older) of 19 346 when the study was begun. About a quarter of the population lives in the main village (A-Estrada, 42°40'N/8°30'W), and the remainder live in a rural environment. An age-stratified random sampling of the population 18 years and older was drawn from the National Health System Registry, which covers more than 95% of the population. The age strata were as follows: 18–29 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, 70–79 years, 80 years and older. A computer program generated a random sample of equal number ($n = 100$) of subjects in each age group, except for the first group (18–29 years), which included 120 subjects. These 720 subjects were invited to participate in the study by a personal letter. From this initial sample, those unable to give informed consent ($n = 23$) were considered ineligible. From the remaining eligible 697 subjects, a total of 469 participated in the study (participation rate, 67.2%). Median age of the participants was 54 years (range: 18–92) of which 206 were males (44.5%). Most participants lived in a rural environment (352 subjects, 75.0%), and the remainder lived in A-Estrada village. There were no significant differences in age, gender, and habitat (rural/urban) between subjects who participated in the study and those who did not. A total of 136 subjects (28.9%) worked as farmers. Educational level was as follows: no studies in 58 subjects (12.3%), elementary studies (1–8 years) in 310 subjects (66.0%), secondary studies (9–13 years) in 70 subjects (14.9%), and university studies in 27 subjects (5.7%). From February to December 2000, all subjects were successively conveyed to the Primary Care Centre for evaluation, including, skin prick tests (SPT) to a standard battery of aeroallergens, blood sampling, and a structured questionnaire.

Allergic sensitization

A panel of SPT to relevant aeroallergens in the area was performed in all subjects. It included mites (*Dermatophagoides pteronyssinus*, *Lepidoglyphus destructor*, and *Tyrophagus putrescentiae*), pollens (*Lolium perenne*, *Plantago lanceolata*, *Betula alba*, and *Parietaria judaica*), molds (*Alternaria alternata*, *Aspergillus spp.*, *Penicillium notatum*, and *Cladosporium herbarum*), and animal danders (dog and cat) (ALK-Abelló, Madrid, Spain). Control SPT included 10 mg/ml histamine and saline solution. Standard procedures were followed (18). Wheals > 3 mm after 15 min were considered positive. The presence of at least a positive SPT was considered indicative of allergic sensitization (19). Mites and pollens were the most common aeroallergens. A detailed description of the profile of allergic sensitization in this study population has been published elsewhere (17).

Hepatitis A virus antibodies

A blood sample could be obtained in 465 of 469 subjects (99%). Serum samples were stored at -20°C until tested for the presence of total HAV serum antibodies by means of a commercially available qualitative enzymeimmunoassay (EIA) test (DiaSorin SA, Madrid, Spain). Patients with a positive test were considered to have HAV exposure.

Symptom questionnaire

A history of upper respiratory symptoms was investigated by means of questionnaire. Subjects were classified as symptomatic when answering 'yes' to any of the following questions: (a) 'have you ever had a problem with sneezing, or a runny, or a blocked nose when you did not have a cold or the flu?', and (b) 'have you ever had wheezing or whistling in the chest at any time in the past'? Subjects with both evidence of allergic sensitization and a history of respiratory symptoms were considered atopic (19).

Statistical analysis

In order to account for the stratified sampling, a design-based analysis was performed. The sampling procedures used in the study departed from unequal probability selection. Compensatory weights were developed to obtain estimates [prevalences and odds ratios (OR), with their corresponding 95% confidence intervals (CI)] from the original target population in the study area (20). The STATA 7.0 (Stata Corp., College Station, TX, USA) statistical package was employed. Cross-tabulation significance levels were based on second-order corrected Pearson chi-square test for categorical variables. For multivariate analysis (logistic regression), age (in years) entered the equation as a quantitative variable. The remaining variables were categorical and entered the equation as '1' ('present' or 'yes') or '0' ('absent' or 'no'). Three dummy variables were used to code the four-category variable 'level of studies', with the lowest category as the reference.

Ethical considerations

The study conformed to the principles of the Helsinki's declaration, and was reviewed and approved by the local Research Committee.

Results

Serologic evidence of HAV exposure was present in 405 subjects (unweighted proportion 87.1%; weighted proportion 83.6%, 95% CI: 80.7–86.5). The HAV exposure was inversely associated with allergic sensitization. The proportion of subjects with allergic sensitization was lower in cases with HAV exposure than in cases without HAV exposure (unweighted proportions 23.5% vs 41.7%; weighted proportions 25.0% vs 43.0%; OR 0.44, 95% CI: 0.25–0.77, $P = 0.004$).

Age was closely associated with both HAV exposure and allergic sensitization (Fig. 1). Age (in years) was positively associated with HAV exposure (OR 1.15, 95% CI: 1.10–1.19, $P < 0.001$). The proportion of cases with HAV exposure steadily increased from 40.0% (95% CI: 28.5–51.4) in the 18–29 year age group to nearly 100% in subjects aged 50 years and older. Only two subjects from the latter age group had no serologic evidence of HAV exposure (Fig. 1). Also, age (in years) was inversely associated with allergic sensitization (OR 0.96, 95% CI: 0.95–0.98, $P < 0.001$). The proportion of cases with allergic sensitization steadily decreased from 54.9% (95% CI: 43.3–66.5) in the 18–29 year age group to 12.8%

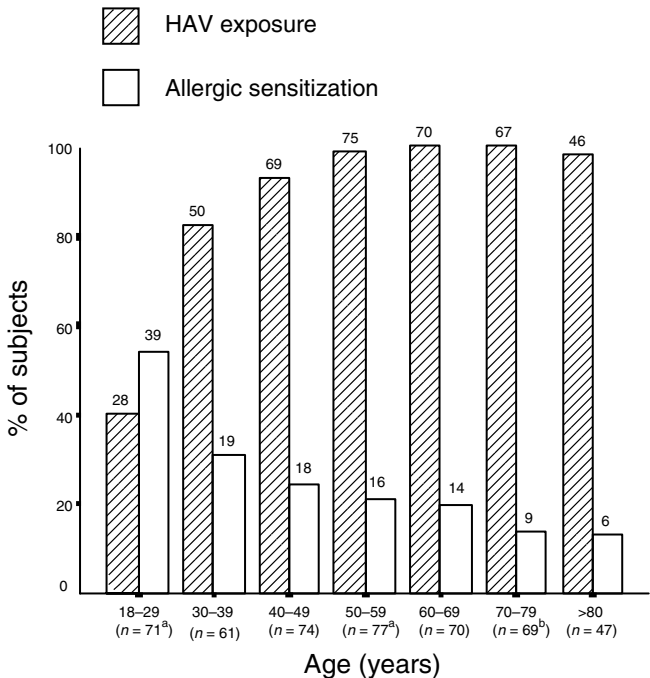


Figure 1. Prevalence of hepatitis A virus (HAV) exposure and allergic sensitization according to age strata. Figures on the top of bars represent the absolute number of subjects. ^aHAV assay was not available in one case of the stratum. ^bHAV assay was not available in two cases of the stratum.

(95% CI: 3.2–22.3) in subjects aged 80 years and older (Fig. 1). The inverse association between age and allergic sensitization was evident in subjects with HAV exposure (OR 0.97, 95% CI: 0.95–0.98, $P < 0.001$) and in patients without HAV exposure (OR 0.89, 95% CI: 0.79–0.99, $P = 0.04$).

The inverse association between HAV exposure and allergic sensitization was substantially altered after adjusting for age (Table 1). When age and HAV exposure were introduced in a multivariate analysis, age maintained the significant association with allergic sensitization, but the association of HAV exposure with allergic sensitization became lower and not significant. Interaction terms were not significant ($P = 0.154$). There was no significant association between HAV exposure and allergic sensitization within any of age strata. Further adjustment for sex, setting (rural/urban), occupation (farming/other), and level of studies did not modify the OR for the association of HAV exposure with allergic sensitization (Table 1).

Similar results were observed when distinct groups of aeroallergen sensitization were considered separately. The HAV exposure was inversely associated with sensitization to mites (OR 0.41, 95% CI: 0.23–0.72, $P = 0.002$), but the association disappeared after adjusting for age (OR 1.08, 95% CI: 0.57–2.08, $P = 0.80$). Also, HAV exposure was inversely associated with sensitization to

Table 1. Relationship of allergic sensitization with hepatitis A virus (HAV) exposure (logistic regression): (a) crude analysis, (b) after adjusting for age, and (c) after adjusting for additional factors

Factor	Allergic sensitization, OR (95% CI)	P-value
(a)		
HAV exposure	0.44 (0.25–0.77)	0.004
(b)		
HAV exposure	1.15 (0.60–2.19)	0.66
Age (years)	0.96 (0.95–0.98)	<0.001
(c)		
HAV exposure	1.19 (0.61–2.31)	0.60
Age (years)	0.96 (0.94–0.97)	<0.001
Sex (male)	1.06 (0.66–1.72)	0.79
Occupation (farming)	0.99 (0.52–1.89)	0.98
Habitat (rural)	0.89 (0.53–1.51)	0.53
Level of studies		
None	1 (reference)	
Elementary	0.71 (0.30–1.65)	0.42
Secondary	0.39 (0.13–1.20)	0.10
University	1.46 (0.44–4.93)	0.54

pollens (OR 0.45, 95% CI: 0.21–0.94, $P = 0.03$), but the association disappeared after adjusting for age (OR 0.98, 95% CI: 0.42–2.27, $P = 0.95$).

Likewise, there was an inverse association between HAV exposure and atopy (symptomatic allergic sensitization). The proportion of atopics tended to be lower in subjects with HAV exposure than in subjects without HAV exposure (unweighted proportions 15.3% vs 25.0%; weighted proportions 16.4% vs 25.9%; OR 0.56, 95% CI: 0.29–1.07, $P = 0.08$). Again, such an inverse association between HAV exposure and atopy disappeared after adjusting for age (OR 1.06, 95% CI: 0.50–2.21, $P = 0.88$).

Discussion

In the studied population, there is an inverse association between HAV exposure and both allergic sensitization and atopy, but this association becomes substantially altered after adjusting for age. This is in apparent contrast with previous studies (summarized in Table 2) that, with few exceptions (21, 22), reported a consistent association between HAV exposure and protection from allergic sensitization (7–11), even after adjusting for potential confounders (7–11). Furthermore, some of these studies have shown an association between HAV exposure and protection from hay fever and/or asthma (8–11).

Particular characteristics of the studied population should be taken into account. First, the overall prevalence of HAV exposure in the present study was very high. Similarly to the Spanish general adult population (23, 24), 40% of young (18–29 year old) adults were exposed to HAV and a nearly 100% plateau of exposure was reached at 50 years. This high prevalence is intermediate between developing countries where the 100% plateau is almost

Table 2. Summary of studies investigating the relationship between hepatitis A virus (HAV) exposure and allergic sensitization

Authors	Study design	Setting and study population	Number of subjects*	Age range (years)	HAV prevalence (%)†	Evaluation of allergic sensitization	Protection of allergic sensitization in HAV-exposed
Matricardi et al. (7)	Cross-sectional	Italy, military students	1659	17–24	27	SPT and specific IgE	Present in crude analysis for SPT (OR 0.64, 95% CI: 0.49–0.84) and for specific IgE (OR 0.47, 95% CI: 0.33–0.68). Present for specific IgE after adjusting for age, sex, and family history of atopy (OR 0.50, 95% CI: 0.35–0.73)
Matricardi et al. (8)	Cross-sectional	San Marino (Italy), general population	1527	20–85	65 (29–97)‡	Specific IgE	Present in crude analysis (OR 0.40, 95% CI: 0.28–0.57)§ and after adjusting for age and sex (OR 0.59, 95% CI: 0.38–0.92)
Bodner et al. (21)	Nested case-control	Grampian region (Scotland), general population	275	39–45	60¶	SPT, specific IgE or total IgE	Absent in crude analysis (OR 1.58, 95% CI: 0.94–2.64)§ and after adjusting for sex, social class, smoking and family history of atopy (OR 1.6, 95% CI: 0.9–2.6)
Matricardi et al. (10)	Cross-sectional	USA, general population	21 260	6 to >90	(<20 to >80)	SPT	Present in crude analysis for nine of 10 individually studied allergens, but only in subjects younger than 40 years
Linneberg et al. (11)	Nested case-control	Copenhagen (Denmark), general population	1109	15–69	20¶ (2–51)	Specific IgE	Present in crude analysis (OR 0.38, 95% CI: 0.27–0.54)§ and after adjusting for age strata, sex, and family history of atopy (OR 0.57, 95% CI: 0.38–0.86)
Present study	Cross-sectional	A-Estrada (Spain), general population	465	18–92	84 (40–100)	SPT	Present in crude analysis (OR 0.44, 95% CI: 0.25–0.77) but absent after adjusting for age (OR 1.15, 95% CI: 0.60–2.19)

Studies including subjects from prior reports (9) and studies with paucity of data (14, 22) were not included.

* Number of subjects with evaluation of HAV exposure.

† Overall prevalence, and prevalence in the youngest and the oldest age groups, within parentheses (percentages are approximated to entire values).

‡ Data obtained from a source study (30).

§ OR and 95% CI were calculated from reported data.

¶ Overall prevalence was calculated from reported data (estimation may be biased because of the study design).

SPT, skin prick test; IgE, immunoglobulin E; OR, odds ratio; CI, confidence interval.

attained during childhood, and full-developed countries where the overall prevalence is lower and the 100% plateau is either reached at older ages or never reached (10–12). In this respect, it is noteworthy that studies showing a protective effect of HAV exposure for allergic sensitization were performed in areas with lower prevalence of HAV exposure (Table 2). Additional peculiar characteristics of the population studied here include a predominant rural habitat and a high prevalence of mite sensitization (17). Country life may protect from adulthood allergy (25). Mite sensitization is associated with some housing conditions that are linked to low socioeconomic status (26). However, a similar inverse association between HAV exposure and sensitization to either mites or pollens was observed. In this respect, it should be noted that HAV exposure is only a surrogate marker of the true variable to adjust for (i.e. poor overall hygiene). Unknown peculiarities of the studied population include the genetic background. Recent studies suggest that the protection of HAV exposure against atopy is restricted to individuals with a specific variant in the gene that encodes

TIM1, the cell surface receptor used by HAV to infect human cells (14).

The number of subjects included in the current study is lower than that included in other studies, but the power was enough to detect a significant inverse association between HAV exposure and allergic sensitization. However, the most remarkable finding of the present study is the confounding effect of age. The broad age of subjects included (18–92 years) and, particularly, age-stratified sampling offers advantages for evaluation of age-related phenomena such as allergic sensitization and HAV exposure. In strict terms, age met all criteria to be considered a confounder in the association of HAV exposure and allergic sensitization (27, 28). First, age was negatively associated with allergic sensitization, even in subjects without HAV exposure. Secondly, HAV exposure was associated with age. Finally, age is not an intermediate variable between HAV exposure and allergic sensitization (27, 28). The substantial change of the OR for the association between HAV exposure and allergic sensitization after adjusting for age further indicates a

confounding effect. It should be noted, however, that the confounding variable (i.e. age) was so strongly correlated with exposure (i.e. HAV) that some degree of collinearity may arise (28). Moreover, HAV exposure in the studied population was nearly a constant from a certain age onwards. Such an insufficient variability could preclude adequate adjustment. Of interest in this regard is a study in Ethiopia (a developing country with high HAV exposure) showing no association between HAV exposure and asthma symptoms (29). In similar populations, age tends to be *per se* a surrogate marker of infection exposure. On the contrary, it should be noted that a partial confounding effect of age in the inverse association of HAV exposure and allergic sensitization is not entirely new. The specific effect of age-adjusting was not given in previous studies, but there are reasons to infer that age may be a partial confounder in the inverse association of HAV exposure and allergic sensitization and therefore explain part of such an association. Adjusting for age and additional factors induced a substantial (nearly 50%) modification of the OR for such an association in previous studies (8, 11). Of note, the inverse association of HAV exposure and allergic sensitization was only significant in subjects younger than 40 years in the largest study investigating the question (10). Adjusting by broad age strata raises the possibility of residual confounding in some studies (11). Narrow age ranges prevent from true

age-adjusting in other studies, although they simply demonstrate that the observed inverse association cannot be solely explained by a confounding effect of age (7, 9). Finally, variability of past exposure in successive generations (birth cohorts) can distort the apparent association between age and either allergic sensitization or HAV exposure that is observed at given point of time. Such a cohort effect (i.e. a change in the rate of a condition according to year of birth, irrespective of age and calendar time) (28) cannot be ruled out by means of a single cross-sectional approach.

The hygiene hypothesis is supported by a large number of studies showing an inverse association of allergic disorders with a variety of infections (2–4, 7–11, 31). Our results suggest, however, that the effect of age should be carefully taken into account in similar studies. Moreover, in populations with such a high prevalence of HAV infection, the association between HAV exposure and allergic sensitization may not be significant after adjusting for age.

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