

Research articles

HEPATITIS A SEROPREVALENCE IN TUSCANY, ITALY

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Information regarding the current seroprevalence of hepatitis A virus (HAV) is useful for the control of HAV infections. The objective of our study was to evaluate the prevalence of anti-HAV antibodies among children (1-5 years old) and young adults (15-20 years old) in Tuscany, in central Italy. A total of 565 sera were collected in three years 1992, 1998 and 2004, equally distributed between the two age groups. The overall proportion of those that tested positive for anti-HAV antibodies was 8.3%. The proportion of immune children (1-5 years old) statistically significantly increased over the years. The percentage of immune subjects among 15-20-year-old young adults varied over the years, not showing a significant statistical trend, nevertheless our findings indicate that in a low endemicity area, adolescents and young adults are becoming increasingly susceptible to HAV infection. On-going monitoring of immunity to HAV is necessary for detecting trends over time.

Introduction

Hepatitis A is generally an acute, self-limiting liver infection transmitted through the faecal-oral route by a picornavirus, hepatitis A virus (HAV) that occurs worldwide and causes about 1.5 million cases of clinical hepatitis each year [1]. The degree of endemicity is closely related to hygienic and sanitary conditions, the socio-economic level and other development indicators [2]. In recent decades Italy has experienced a declining trend of HAV prevalence [3], probably related to improved health and sanitary conditions which have been responsible for the progressive decline in the infection rate among children under 14 years of age and a major shift towards the highest incidence in susceptible teenagers and young adults [4]. Nevertheless, Italy is considered to be an area with low/intermediate endemicity of hepatitis A. Data from the national surveillance system for acute viral hepatitis infection (Sistema Epidemiologico Integrato dell'Epatite Virale Acuta, SEIEVA), suggest a steady decrease in the incidence of reported cases of HAV infection over the past few years (from 10 per 100,000 inhabitants in 1985 to 3.6 per 100,000 in 2004) [5].

However, the epidemiological situation varies from region to region within Italy [4]. The practice of consuming contaminated raw seafood still causes outbreaks, especially in southern Italy. In particular, Puglia and Campania, two regions in the south, experienced a large outbreak during 1996-7 with approximately 11,000 cases of HAV infection reported, accounting for an annual incidence rate of approximately 130 per 100,000 population [6,7]. Moreover, in 2004 another outbreak, involving 882 cases, was described in Campania [8].

The availability of a safe, highly immunogenic vaccine that provides long-term protection against HAV has been proven to be useful in the containment of hepatitis A in endemic areas [9]. However, high costs and the limited availability of the HAV vaccine have raised some concerns regarding mass vaccination [4,10]. In Italy the current National Vaccination Plan (Piano Nazionale Vaccini PNV 2005-2008), recommends vaccination against HAV only for specific population groups (travellers to endemic areas, drug users, men who have sex with men (MSM), soldiers, sewage workers, patients presenting with liver disease, recipients of liver transplants and HAV-negative haemophiliacs) [11,12]. Since 1998, after a large epidemic of hepatitis A, the Puglia region (south-eastern Italy) has introduced a free-of-charge mass vaccination program (the first ever in Italy since safe and highly effective hepatitis A vaccines became available in 1995) for newborns (15-18 months of age) and adolescents (12 years of age), as part of the routine immunisation schedule, in order to reduce transmission [9]. For this reason, since 2001, when the Italian National Health System was decentralised, the regional health authorities have implemented vaccination strategies according to their own judgment. However, the region of Tuscany does not include hepatitis A vaccination in the regional infant and adolescent immunisation calendar. Preventive hepatitis A vaccination, however, is considered, in Tuscany and all other Italian regions, for close contacts of clinical cases as control measure in case of an epidemic.

Moreover, although hepatitis A is usually a self-limited disease, the likelihood and severity of symptomatic illness are age-related. In a low endemicity area the highest frequency of HAV infection is observed in adults, who are more likely to have clinical symptoms since the infection causes significant morbidity, along with absenteeism, hospitalisations and occasional mortality, while infants and young children are usually asymptomatic [13,14]. In the last decade, in Italy, a progressive reduction of the prevalence of the infection in children, teenagers and young adults has been described. However, the symptomatic/asymptomatic ratio and the percentage of patients with a more severe clinical presentation have progressively increased [15].

The objective of the present study was to determine the prevalence of anti-HAV antibodies in children and young adults in Tuscany, in central Italy, and to present epidemiological data on HAV infection in this area.

Methods

Serum samples

Serum samples from individuals aged 1-5 and 15-20 and collected during three different years: 1992, 1998 and 2004, in the region of Tuscany, were tested. Serum specimens were obtained by using leftover serum from specimens taken for diagnostic purposes and submitted to the Laboratory from individuals who received care in the University Hospital of Siena. The sera collected from the two ranges of age were randomly selected from the laboratory stock. We excluded samples from individuals known to be HIV-seropositive. Samples were collected anonymously and only the age of the subject and the date of the sample were recorded.

A total of 565 sera (283 for the age group 1-5 years and 282 for the age group 15-20 years, approximately 100 samples for each year) were collected and stored at -20°C. Of the 565 serum samples collected all were tested for the anti-HAV-IgG antibody: 47 (8.3%, 95% CI: 6.03-10.57) were positive, 509 (90.1%, 95% CI: 87.64-92.56) were negative and 9 (1.6 % CI: 0.57-2.63) gave ambiguous results. Thus, 556 samples were included in the statistical analysis, as the nine giving ambiguous test results were excluded (Table).

Serological test

Serological testing was performed at the Department of Physiopathology, Experimental Medicine and Public Health of the University of Siena. All serum samples were tested for detection of anti-HAV-IgG antibodies. The antibody index (calculated as sample OD/cut-off serum mean OD x 10), was determined by a commercial enzyme immunoassay test (Enzywell HAV Antibody, DIESSE) following the manufacturer's instructions. According to the manufacturer, the test has a sensitivity and specificity of 100%.

Samples with an index of < 0.8 were considered positive, samples with an index > 1.2 were considered negative, and samples with an index ranging from 0.8 to 1.2 were considered equivocal. Equivocal sera were retested for confirmation.

A positive result for total anti-HAV IgG antibodies is not able to distinguish recent infection from a previous one, thus a test for determining IgM antibodies is necessary for correct detection of current infection. However, the test for determining total anti-HAV IgG antibodies is important since this indicator allows for the identification of cohorts of subjects that are still at risk of infection.

There also is no way of determining whether the positive test results are due to past infection or due to vaccination. Detection of hepatitis A IgG antibodies indicates either past infection or vaccination.

Statistical analysis

Age-specific (1-5 years and 15-20 years) seroprevalence rates were calculated, along with the corresponding 95% confidence intervals (CI). The statistical analysis was performed by Epi Info version 6 program using the chi-squared (χ^2) test, as well as χ^2 test for trend to evaluate possible tendencies. Differences were regarded as significant when $p < 0.05$.

Results

Of the 565 serum samples collected, all were tested for the anti-HAV-IgG antibody: 47 (8.3%, 95% CI: 6.03-10.57) were positive, 509 (90.1%, 95% CI: 87.64-92.56) were negative and 9 (1.6 % CI: 0.57-2.63) gave ambiguous results.

The nine specimens with equivocal results were eliminated from the statistical analysis. Among these, five were taken from 1-5 years old children (four males, one female) and four were from patients aged 15-20 years (two males, two females). Thus, 556 samples were suitable for calculating the results.

Of the 278 serum samples from 1-5-year-old children 26 were positive (9.4%, 95% CI: 6.20-13.40) and of the 278 serum samples from the age group 15-20 years 21 were positive (7.6%, 95% CI: 4.73-11.31).

The percentage of immune children (1-5 years) increased from 2.7% (95% CI: 0.33-9.5) in 1992, to 6.2% (95% CI: 2.30-13.0) in 1998, and to 16.7% (95% CI: 10.19-25.05) in 2004 (Figure). The annual trend was statistically significant ($\chi^2 = 8.9$, p value = 0.0027).

The percentage of immune young adults, aged 15-20 years, varied from 7.5% (95 % CI: 2.80-15.61) in 1992, to 11.3% (95% CI: 5.0-21.0) in 1998, and to 5.5% (95% CI: 2.24-11.03) in 2004 (Figure). In this case, the yearly trend was not statistically significant.

Discussion and conclusion

The results of this study showed an increasing trend of the seroprotection rate in children aged 1-5 years with a particularly high antibodies titre in 2004 (χ^2 for linear trend=10.7; $p=0.0011$). The high anti-HAV antibodies rate among children in 2004 is likely to have been related to extensive vaccination of this age group in consequence of a small epidemics that occurred in communities of children in central Italy during that period [16]: children attending primary schools were contaminated by infected schoolmates who had contracted hepatitis A by eating raw seafood in endemic areas (Campania and Puglia) during Christmas holidays.

One case-control study conducted during an outbreak of hepatitis A which occurred in 2004 in southern Italy and affected different municipalities of Campania region, in the municipality with the highest attack rate showed that raw seafood consumption, particularly when it was illegally stored in water, was strongly associated with HAV infection [8]. The major role played by shellfish consumption in HAV transmission in Italy is supported by data from the surveillance system for type-specific acute viral hepatitis (SEIEVA) [5].

TABLE

Samples tested for the prevalence of antibodies against hepatitis A virus, Tuscany, Italy, by patients' age group, gender and the year of obtaining the specimen

Year of obtaining the sample	Age group (gender: male - female)	
	1-5 years n (m - f)	15-20 years n (m - f)
1992	73 (42-31)	80 (21-59)
1998	97 (44-53)	71 (22-49)
2004	108 (54-54)	127 (65-62)
Total	278 (140-138)	278 (108-170)

On the other hand, the prevalence of anti-HAV antibodies in young adults (15-20 years old) was higher during 1992 and 1998 (7.5% and 11.3%), followed by a decrease to 5.4% in 2004 (χ^2 for linear trend=0.40; $p=0.53$). However, this is not enough to support the presumption that there is an increasing trend in the risk of infection in young adults.

These results must be considered cautiously because the samples studied cover only three years (1992, 1998 and 2004) during a period of eight years (from 1992 to 2004). Nevertheless, these findings confirm the shift of the seroprevalence of hepatitis A virus infection in younger age groups, as observed in the urban population of India [17], in an area with low endemicity where adolescents and young adults are becoming increasingly susceptible to HAV infection.

In the past few years similar studies have been conducted in Italy but only two described the situation in the whole country. However, direct comparisons with these studies are difficult due to the differences in the age groups considered. One, conducted in 1990, showed an anti-HAV immune prevalence of 2.3% among 3 to 5 year-old children, and of 16.3% in teenagers aged 17-19 [18]. The other study performed on sera collected in 1997-1998 showed a prevalence of 34.9%, 12.9% and 14.6% in age groups of 0-1, 2-5 and 12-19 years respectively [19].

Other seroepidemiological investigations have only been conducted in specific areas or among certain risk groups. In 1994 the seroprevalence of HAV antibodies was tested in north-east Italy: the prevalence obtained was 0.7% among the group aged 10-19 years old and 6.0% in the group of over 19 years. Anti-HAV antibodies prevalence in army recruits was 66% in 1981, 30% in 1990 and 5% in 2003 (ranging from 2% in the north to 8% in the south) [20]. Furthermore, a specific report on the prevalence of hepatitis A virus (HAV) in a group of drug users in Italy showed an overall seroprevalence of 28.7% [21].

In conclusion, information regarding the current status of hepatitis A immunity, including the seroepidemiological survey described here, is crucial for providing new and timely parameters useful for mathematical modelling, such as the one proposed and investigated for temporal trends in southern Italy [22]. A

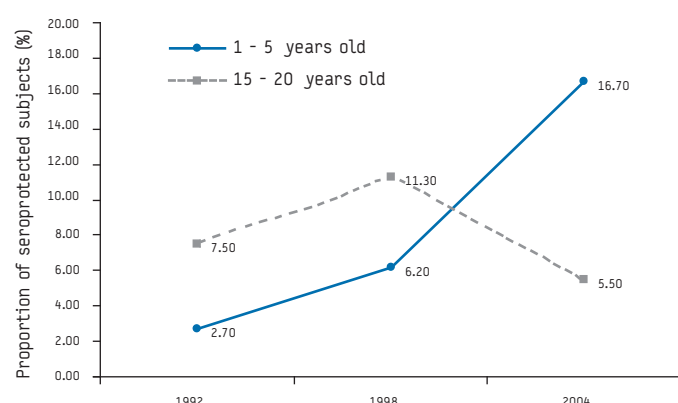
vaccination program for risk groups, travellers and food handlers would be suitable for the control of this virus at present, however, the current epidemiological situation in Italy does not suggest the need for mass vaccination of newborns and adolescents outside the endemic areas, such as Puglia and Campania. The same is true in other southern European countries, such as Spain, where universal immunisation seems economically ineffective and sufficient evidence for targeted vaccination has not yet been gathered [4].

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FIGURE

Seroprevalence of antibodies against hepatitis A virus, in age groups of 1-5 and 15-20 years, Tuscany, Italy, 1992, 1998, 2004



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