

Hepatitis A seroprevalence in a random sample of the Turkish population by simultaneous EPI cluster and comparison with surveys in Turkey

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SUMMARY: Kanra G, Tezcan S, Badur S, Turkish National Study Team. Hepatitis A seroprevalence in a random sample of the Turkish population by simultaneous EPI cluster and comparison with surveys in Turkey. *Turk J Pediatr* 2002; 44: 204-210.

This study was conducted to determine the hepatitis A virus (HAV) seroprevalence in nine provinces representative of Turkey as a whole. These provinces are representative of the country's geographical location, and demographic, economic and social characteristics. In each province, sample sizes were determined using published data on HAV seroprevalence, and sample sizes for each province and for the cluster were calculated for each group of subjects under the age of 30 for seroprevalence estimates within a 95% confidence interval. The samples were selected by a cluster method, and the planned recruitment was a total of 4,800 subjects, including 600 subjects each from five large provinces (İstanbul, Ankara, İzmir, Adana, Diyarbakır) and 450 subjects from each of the remaining four provinces (Samsun, Erzurum, Trabzon, Edirne). These numbers were distributed in accordance with the percentages for age groups in five-year increments starting from age five for the population under the age of 30 living in the rural and urban areas in each province. This study of 4,462 subjects under the age of 30 in nine provinces of Turkey identified an overall HAV seroprevalence rate of 71.3%. The distribution of HAV seroprevalences by age showed a steady increase from one year of age from 42.7% to 91.1% at 25-29 years of age. HAV seroprevalence was slightly higher in female subjects (73%) than in male subjects (69.3%). By educational status, seroprevalences were comparable except in young children under age six. Seroprevalence was notably higher in large families with six and more members (80.1%) than in small families with five or fewer members (66.7%). According to our study results, 50% of Turkish children are seropositive for HAV by the age of 10 years. We believe the data support the need for a routine primary immunization policy in Turkey and the development of effective prophylactic programs after possible exposure. Consequently, an immunization policy can be developed for each region according to its epidemiological conditions.

Key words: hepatitis A, vaccine, seroprevalence, Turkey.

Hepatitis A virus (HAV) is an endemic infectious disease caused by a picornavirus and transmitted by fecal-oral route¹.

The infectious spectrum of the disease is broad. Jaundice, the most important symptom, is absent or mild, leading to underdiagnosis or missed diagnosis in young children¹. Moreover, the Ministry of Health often does not receive correct notification of diagnosed HAV cases; the Ministry also includes all types of hepatitis under one title in the list of diseases requiring notification. Thus, the actual number of hepatitis A cases in Turkey is not known.

Various studies about hepatitis A seroprevalence have been conducted in Turkey. However, these studies included only a limited number of patients in small localization, not representing the entire country. In a study by Coşkun et al.² that included 287 subjects in İzmir, anti-HAV seroprevalence was found to be 96% in the general population and 97.4% in those above age 15. In a study with 468 subjects living in İstanbul, Babacan et al.³ found prevalence rates of 67% in the general population and 73.7% in those above age 15, respectively. In another study, Taşyaran et al.⁴ demonstrated that anti-HAV seroprevalence was 33.3% in children between 3-6 years, 78.6% in those of 7-10 years, and 77.5% in the age group of 11-14 years in the province of Erzurum. Also, Badur et al.⁵ reported a 98.3% HAV seroprevalence rate among blood donors in Diyarbakır.

The lack of a nationwide study identifying the HAV seroprevalence in the general population in Turkey impedes public health planning of a vaccine program and prophylactic measures after possible contact. We conducted this study to determine HAV seroprevalence in nine provinces representative of Turkey, as a whole.

Material and Methods

This HAV seroprevalence study was conducted in nine provinces of Turkey, taking into account the urban and rural areas of each province. These provinces are representative of the country's geographical location and demographic, economic and social characteristics. One-third of Turkey's population live in these nine provinces. Moreover, the population in these areas also includes a high number of residents who have migrated from neighboring and outlying provinces. The

selected provinces were İstanbul, Ankara, İzmir, Adana, Diyarbakır, Samsun, Trabzon, Erzurum and Edirne.

We obtained the population distribution by rural and urban areas in each province from the results of the 1997 General Census conducted by the State Institute of Statistics⁶. Considering the age-related results of various local seroprevalence studies on the housing and environmental conditions in Turkey, we decided that a study of the population under age 30 would be most appropriate for investigation seroprevalence and certain epidemiological characteristics³⁻⁵ of HAV.

In each province, sample sizes were determined using published data on HAV seroprevalence, and sample sizes for each province and for the cluster were calculated for each group of subjects under age 30 for seroprevalence estimates within a 95% confidence interval⁷. The planned recruitment was a total of 4,800 subjects, which included 600 subjects each from five large provinces (İstanbul, Ankara, İzmir, Adana, Diyarbakır) and 450 subjects from each of the remaining four provinces (Samsun, Erzurum, Trabzon, Edirne). These numbers were distributed in accordance with the percentages for age groups in five-year increments for the population under the age of 30 living in the rural and urban areas in each province. The study was conducted between February and December 1998. The sampling method of 30 clusters recommended for field studies by the World Health Organization was used. This provided a practical method for selecting subjects of a pre-determined number in the rural and urban areas in each province⁸. A total of 60 clusters were chosen, equally divided between urban and rural areas.

All subjects who were interviewed and who provided blood samples completed a written informed consent form. To obtain epidemiological data, subjects completed a questionnaire on housing conditions and several economic variables. Blood samples obtained from each subject were centrifuged and serum samples were stored at -20 °C. In each center, investigators were appropriately trained on issues such as the selection of subjects, obtaining informed consent, applying the questionnaire, taking blood samples, and collecting, storing and transporting sera for the

serum analysis in order to provide a standardized practice. Additionally, the authorized Ethics Committee in each center approved the seroprevalence study and the Central Ethics Committee of the Ministry of Health approved the entire study.

Serum samples were obtained and transported to the related laboratory in accordance with the principles of cold chain. For each serum sample, a quantitative investigation for anti-HAV-IgG antibodies was performed using Dade-Behring micro-ELISA kits (Dade Behring Marburg, Germany).

All serum samples including controls and references were diluted by 1/10 and added to two separate aliquots, one containing viral antigen and the other containing control antigen. Following an incubation period of 60 minutes at 37 °C, aliquots were irrigated three times so that the unbound materials were removed, then peroxidase-labelled anti-human conjugate (100 µl) was added. Following an incubation period of 60 minutes at 37 °C, aliquots were irrigated again, and the chromogen substrate was added. The reaction occurring in ELISA plates stored at room temperature for 30 minutes was terminated by adding 100 µl of stopper buffer solution to each aliquot. Subsequently, absorbency value for each aliquot was detected by reading at 450 nm. By considering the absorbency values for aliquot covered with or without antigen and using α -method, anti-HAV-IgG level was quantitatively determined for each serum sample.

As this is a descriptive study, no statistical analysis was performed and only the percentages were given.

Results

Although 4,800 subjects were planned, only 4,462 (93%) subjects were interviewed and underwent blood sampling. The overall HAV seroprevalence rate among these subjects was 71.3% Table I shows the distribution of subjects by province who completed the questionnaire and underwent blood sampling.

In Ankara, İzmir and Erzurum, investigators questioned and obtained blood samples from more subjects than the suggested target sample size. Seroprevalence could not be determined in 7% of selected subjects and 5.1% of questioned subjects. Blood samples could not be obtained from some children under the age of one year due to the difficulty of obtaining blood sample.

Table II shows the demographic characteristics of the subjects and also shows the overall distribution of HAV seroprevalences by age groups, steadily increasing from one year of age from 42.7% to 91.1% at 25-29 years of age. The majority of those living in the study provinces had been infected with HAV before age 20. HAV seroprevalence was slightly higher in female subjects (73%) than in male subjects (69%), but the difference was not significant. By educational status, seroprevalences were comparable except in young children under age 6 (Table II).

Table I. Distribution of subjects from nine provinces selected for sampling, who were interviewed and underwent blood analysis (Turkey 1998)

Provinces	Number of sampled subjects	Number of interviewed subjects		Number of subjects who underwent blood sampling	
		Number	%*	Number	%**
İstanbul	600	563	93.8	558	99.1
Ankara	600	636	106.0	624	98.1
İzmir	600	627	104.5	617	97.6
Adana	600	570	95.0	547	96.0
Diyarbakır	600	540	90.0	470	87.0
Samsun	450	430	95.6	347	80.1
Erzurum	450	569	126.4	564	99.1
Trabzon	450	387	86.0	376	97.2
Edirne	450	379	84.2	359	94.7
Total	4800	4701	97.9	4462	94.9

* Percentages calculated for the number of sampled subjects in each province.

** Percentages calculated for the number of interviewed subjects in each province.

Table II. Distribution of subjects under the age of 30 from nine provinces by several demographic characteristics (Turkey 1998)

Characteristic	Number	Percentage	HAV Seroprevalence
Age			
0	61	1.3	70.2
1-4	727	15.5	42.7
5-9	875	18.6	57.0
10-14	902	19.1	70.6
15-19	798	17.0	82.5
20-24	670	14.3	90.9
25-29	595	12.7	91.1
Unknown	73	1.5	87.3
Sex			
Male	2260	48.1	69.2
Female	2441	51.9	73.1
Education			
Child under 6	1025	21.8	49.7
Student	1581	33.7	85.8
Literate*	90	1.9	94.0
Primary School	899	19.4	85.6
Secondary-High school	783	16.9	85.6
University	95	2.1	87.9
Unknown	197	4.2	73.9
Type of Location			
Urban	3074	65.7	69.9
Suburban**	300	2.1	67.3
Unknown	4		
Family size			
≤ 5	3081	65.5	66.7
Six and more ≥ 6	1620	34.5	80.1
Total	4701	100.0	

* Able to read and write.

** Residential areas around the cities and beyond the official municipality boundaries.

HAV: hepatitis A virus

Family size did have a significant impact on HAV seroprevalence (Table II). Seroprevalence was notably higher in large families with six and more members (80.1%) than in small families with five or fewer members (66.7%). Seroprevalence also differed with housing conditions. Subjects living in houses with tap water (70.0%) or toilet (70.1%) had a significantly lower rate of seroprevalence than in those without these facilities (84.3% in houses with no tap water and 77.1% in houses with no indoor toilet).

Figure 1 shows the distribution of seroprevalence rates by age. Except for Edirne, seroprevalence showed an increase from the age of five years up to age 20, whereas such increase declined as natural immunity developed in the population at later ages. Seroprevalence was also higher in adolescents in Eastern rather than

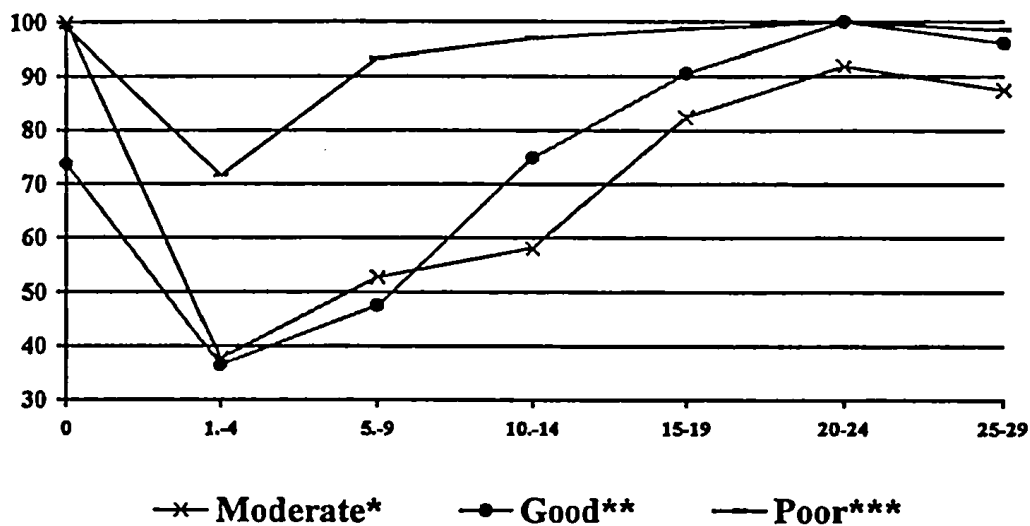
Western provinces. In Turkey, economic conditions are better in the west than the east. In the relatively richer portion of Turkey, HAV seropositivity developed later, more profoundly in the adolescent age group.

Discussion

Hepatitis A infection is more likely to be asymptomatic during childhood; infection at an older age increases the likelihood of symptom development of clinical diseases, especially jaundice⁹.

Anti-hepatitis A virus IgG (anti-HAV IgG) has been accepted as a marker for previous hepatitis A infection and provides immunity against the disease⁹⁻¹¹.

Results from epidemiological studies conducted in other countries demonstrate that the presence of hepatitis A antibody can vary



* Adana, Edirne, Trabzon, Samsun.

** Ankara, İstanbul, İzmir.

*** Erzurum, Diyarbakır.

HAV: hepatitis A virus.

Fig. 1. Age-specific HAV seroprevalence in Turkey according to socioeconomic condition of the provinces.

between different regions and ages^{10,13,14}. Independent of sex and race, these differences may be due to local conditions. Size and heterogeneity of the study groups also may influence results. Representative seroepidemiological data is needed to establish preventive vaccination programs as well as a post-exposure prophylactic therapy regimen¹¹.

This is the first large seroprevalence study in Turkey. Most other studies were conducted with a limited number of subjects who were blood donors or had been admitted to the hospital^{10,11,13-16}. Based on the population demographics of the 1997 general census, our study subjects served as a representative cross sample for the entire population under the age of 30 in Turkey.

Our study included a total of 4,462 subjects under the age of 30 from different provinces, with 93% of the targeted sample size being reached. This is the largest seroprevalence study in Turkey. The overall seroprevalence of hepatitis A was found to be 71.3%.

As HAV seroprevalence was 70.2% in infants under one year of age and 73.2% in women under the age of 30, we concluded that the transmission of maternal antibodies occurs at a high rate. Although the sample size was small, this finding supports our findings from earlier studies where seroprevalence was determined

to be high^{10,11,13-14}. The fact that the seroprevalence rate decreased to 42% between the ages of 1-4 years despite contact with hepatitis A suggests that the high level of HAV seroprevalence is caused by maternal antibodies in children under one year of age. Such a high level will cause a decrease in the success rate of vaccination during the first year, as demonstrated by Kanra et al¹⁷.

The high level of maternal antibodies (up to 70%) in the first year of life and the strong possibility of a high rate of contact with hepatitis A due to the high level of endemicity must be considered when planning a vaccination program. For example, in the U.S. hepatitis A vaccination after 24 months as been recommended since the year 2000 in states with a high incidence rate of hepatitis A¹⁸.

In our study, HAV seroprevalence increased in subjects from ages one to four years, and the increase continued during the adolescent period. These results demonstrated a high risk of hepatitis A infection in schoolage children and adolescents in Turkey.

Crowded conditions and close personal contact are also important risk factors for hepatitis A infection and are common in schools.

Our study showed no difference in HAV between the sexes, as supported by other studies^{12,13,15,16}.

In the study by Szmuness et al¹⁶. in New York City, the level of hepatitis A antibodies was shown to be two- or three-fold higher in subjects with lower socioeconomic conditions than in those with a higher level of welfare and better living conditions.

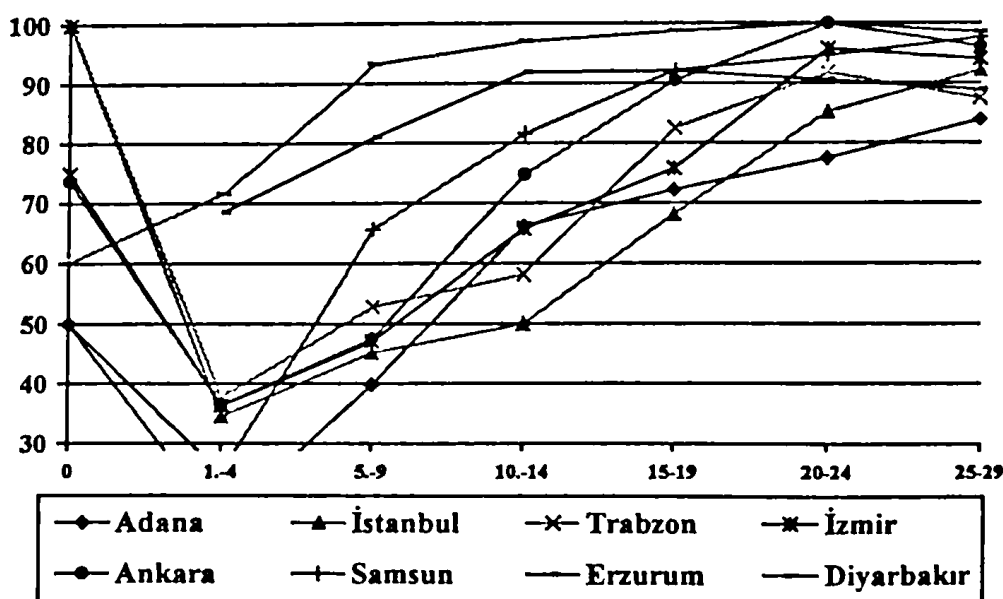
In comparison of provinces, seropositivity was significantly lower in Adana (56.7%) with a high level of wealth and in Edirne (60.7%) with a high level of education and socioeconomic development than in the poor provinces of Diyarbakır (91.9%), Erzurum (85.1%) and Samsun (83.6%) (Fig. 2). The higher seropositivity in these provinces may be due to rapid, unplanned development with poor infrastructure. These provinces also have a high level of migration from the rural areas or neighboring provinces. Seropositivity was generally lower in subjects living in houses with tap water and toilet than in those without these facilities, suggesting that unplanned development without adequate sanitary infrastructure may be a risk factor for the disease¹⁵. As expected, contact with hepatitis A seemed more likely in large families. This is not surprising if we accept that the higher number of family members is inversely correlated with the welfare level and socio-cultural development of the family. In Ankara, İstanbul and İzmir, however, where infrastructure services are relatively sufficient,

even in the suburbs, the family size had no effect on the seropositivity.

The first step in preventing hepatitis A infection is to improve the personal hygiene and infrastructure across the country. The results show that hepatitis A is moderately endemic in Turkey although seroprevalence differs from one region to another. The World Health Organization (WHO) defines, in general, moderately endemic countries as those developing, having a transitional economy, and having industrialized regions. In these countries, children are infected with hepatitis A at early ages. Additionally, viral spread is high, and the infection is more frequently seen in adolescents and young adults. The infection may be spread through personal contact, although outbreaks traced to contaminated water and food may result from inadequate infrastructure. The disease follows a course of successive cycles, and outbreaks occur when a sufficient number of sensitive people are reached, and thus almost all the people are at risk.

Turkey fits the WHO profile of a developing country where HAV exposure begins during childhood and increases with age. Studies have shown that vaccination plays an important role in preventing outbreaks in these countries.

According to our study results, 50% of Turkish children are seropositive for HAV by the age of



HAV: hepatitis A virus.

Fig. 2. HAV seroprevalence according to age throughout Turkey.

10 years. In addition, seropositivity is not similar across provinces of Turkey. HAV vaccine is recommended in areas with high endemicity in some developed countries. In the year 2001, the routine immunization schedule included HAV vaccine in six states in the U.S. Since there is a big difference in seroprevalence between the provinces in Turkey, a similar program may be recommended in here.

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