

## Levels of *Haemophilus influenzae* type B (Hib) antibody in Turkey before routine immunization

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**Aim:** The *Haemophilus influenzae* type b (Hib) vaccine was introduced into the routine immunization schedule in Turkey on January 1, 2007. This study investigates the immunity to Hib prior to the introduction of the vaccine.

**Materials and methods:** Serum samples were collected from healthy volunteers between the ages of 6 months and 50 + years of age, living in the area of 26 health centers in the Samsun, Antalya, and Diyarbakir provinces during 2000-2001. In total, 1713 sera were selected for this study, and the younger age groups were given priority (range 0-82). A commercial ELISA test was used to measure anti-Hib IgG. Hib antibody concentrations of  $\leq 0.15$ ,  $> 0.15$  to  $< 1.0$ , and  $\geq 1.0$   $\mu\text{g/mL}$  were considered as non protective, short-term protection, and long term protection, respectively. The statistical analyses performed were the chi-square test and logistic regression.

**Results:** The antibody levels considered non-protective were 58.0%, 35.3%, 28.1%, 19.8%, 18.4%, 15.3%, and 20.6% among  $< 5$ , 5-9, 10-19, 20-29, 30-39, 40-49, and 50 and over age groups, respectively. Protection against Hib infection increased by age in all 3 provinces.

**Conclusion:** This study provides important baseline data on anti-Hib antibody levels in Turkey before routine immunization was introduced, and shows that there is a considerable percentage of subjects with non-protective antibody levels.

**Key words:** *Haemophilus influenzae* type b, antibody, serosurveillance

### Türkiye'de rutin immunizasyon öncesi *Haemophilus influenzae* tip B (Hib) antikor düzeyleri

**Amaç:** Türkiye'de 1 Ocak 2007 tarihinden itibaren *Haemophilus influenzae* tip b (Hib) aşısı rutin immünizasyon programına dahil edilmiştir. Bu çalışma, aşılamaya öncesinde Hib' e karşı bağışıklık durumu hakkında fikir vermektedir.

**Yöntem ve gereç:** Serum örnekleri 2000-2001 yılları arasında Samsun, Antalya and Diyarbakir illerinde yaşayan 26 sağlık merkezi tarafından 6 ay ila 50+ yaş arası gönüllü sağlıklı kişiler arasından toplanmıştır. Bu serumlardan 1713'ü bu çalışmada test edilmek üzere seçilmiştir ve genç yaş gruplarına öncelik verilmiştir (0-82 aralığında). Anti Hib IgG antikorlarını ölçmek için ticari bir ELISA testi kullanılmıştır. Koruyucu olmayan düzey  $\leq 0,15$   $\mu\text{g/mL}$ ; kısa süreli korunma  $>0,15$ - $<1,0$   $\mu\text{g/mL}$  ve uzun süreli koruyucu titre  $\geq 1,0$   $\mu\text{g/mL}$  olarak kabul edilmiştir. İstatistiksel analizler için Ki-kare ve lojistik regresyon testleri kullanılmıştır.

**Bulgular:** Yaş grupları  $<5$ ; 5-9; 10-19; 20-29; 30-39; 40-49; 50 ve üzeri için koruyucu olmayan antikor titreleri sırasıyla % 58,0; % 35,3; % 28,1; % 19,8; % 18,4; % 15,3 ve % 20,6 olarak bulunmuştur. Her üç ilde de Hib enfeksiyonuna karşı korunma yaş ile birlikte artmaktadır.

**Sonuç:** Bu çalışma rutin aşılamaya öncesinde Türkiye'de anti-Hib antikor düzeyleri hakkında önemli veri sağlamaktadır ve ayrıca koruyucu olmayan antikor seviyesine sahip kişilerin önemli bir yüzde oluşturduğunu göstermektedir.

**Anahtar sözcükler:** *Haemophilus influenzae* tip b, antikor, serosurveyans

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## Introduction

It is estimated there are 3 million cases and 386,000 deaths globally each year associated with severe *Haemophilus influenzae* type b (Hib) infection with the greatest burden in developing countries. Hib-associated diseases in children are primarily meningitis in children between 3 months and 3 years of age, and epiglottitis and other invasive diseases, such as pneumonia, in children 2 to 4 years of age. A conjugate Hib vaccine, consisting of polyribosylribitol phosphate (PRP) as an antigen and a protein immunogen, is now used routinely in more than 90 countries. The use of this efficacious and reliable vaccine has dramatically reduced the incidence of Hib-associated disease. In countries that have achieved high immunization coverage, possibly as a result of inducing herd immunity, nasopharyngeal carriage has been diminished, and the transmission by droplet has decreased (1,2).

A conjugated Hib vaccine was introduced into the routine immunization schedule of Turkey in January 2007. The vaccine is currently given in conjunction with DPT-IPV in the 2nd, 3rd, and 4th months, with a booster dose given between 16 and 24 months of age (3). This study investigated the immunity to Hib following natural exposure to the microorganism, before vaccination. The aim of this study was to provide baseline data for use in monitoring the impact of the introduction of the Hib vaccine into the routine immunization schedule.

## Materials and methods

### Study group

During the Japanese International Cooperation Agency (JICA) funded "Infectious Diseases Control Project in Turkey", a total of 2465 serum samples were collected from 3 provinces in Turkey (4). Before collecting the serum samples, signed consent forms were collected from all participants. For those under 18 years of age, consent was given by their parents. Study participants were selected randomly from healthy subjects living in the areas of 26 health centers. Epidemiological and demographic data were collected through a questionnaire-based interview and subjects were given a basic physical examination before blood was collected. The age groups were

designed for 16 individuals per age group, per survey, whereas gender and location were equally distributed. The provinces were selected from different geographic regions and socioeconomical levels of Turkey. Of these, 1713 were included in this study to be cost effective. The distribution of sera selected for this study gave priority to younger age groups by selecting 74, 79, 84, 77, 81, 85, 75, 65, 75, and 65 serum samples from 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 years of age, respectively. For analysis, age groups were arranged into 0-4, 5-9, 10-19, 20-29, 30-39, 40-49, and 50 + year-old group (mean  $22.66 \pm 20.43$ , range 0-82) and the numbers of serum samples tested were 395, 365, 160, 197, 179, 170, and 247, respectively. In this study, 524 (30.6%) of the study participants were from Diyarbakır, 580 (33.9%) from Samsun, and 609 (35.6%) from Antalya. There was no significant difference between provinces when age, gender, or location (rural/urban) were considered (respectively;  $P = 0.388, 0.530, 0.121$ ).

Serum samples were collected by obtaining venous blood, were shipped by cold chain, and kept at  $-80^{\circ}\text{C}$  until analysed.

### Laboratory tests

A commercial ELISA (Immunozyg Hib IgG, Progen Biotechnik GmhB, Heidelberg, Germany) was used to measure anti-Hib IgG antibodies against polyribosylribitol-phosphate (PRP) of *Haemophilus influenzae* type b in human serum, in Refik Saydam National Hygiene Center, Communicable Diseases Research Department. Testing of serum samples was carried out according to the manufacturer's recommendations, and the optical densities of test reactions read at 405 nm. Standard sera were used to generate a calibration curve for each test batch, and this was used to calculate the antibody concentration from the measured optical density (OD). For quality control purposes, standard sera, positive high level, and positive low-level control's OD were used. The lowest antibody concentration detectable was considered to be  $0.1 \mu\text{g/mL}$ .

Hib antibody concentrations  $\leq 0.15 \mu\text{g/mL}$  were considered as non-protective,  $> 0.15 \mu\text{g/mL}$  to  $< 1.0 \mu\text{g/mL}$  were considered to indicate short-term protection, and  $\geq 1.0 \mu\text{g/mL}$  indicated long term protection (1,5,6). Serum samples were grouped according to these levels, and 14, 8, and 8 (for a total

of 30) serum samples were randomly selected from each level, respectively. These samples were retested at the Health Protection Agency (HPA), Center for Emergency Preparedness and Response Laboratory, UK, using an in-house ELISA.

**Statistical analysis**

The chi-square and one-way ANOVA test were used to evaluate statistical differences between the provinces. The relation between antibody levels and age, gender, and location was assessed by chi-square and logistic regression analysis. For logistic regression (LR) analysis indicative protective ( $\geq 1.0 \mu\text{g/mL}$ ) and non-protective ( $< 1.0 \mu\text{g/mL}$ ) values were accepted as dependent variables and odds ratios (OR) were calculated using a 95% confidence interval (CI). SPSS for Windows 15.00 was used for the analysis.

**Results**

Results obtained using the commercial ELISA and the HPA in-house ELISA demonstrated good correlation, with a correlation coefficient of 0.87.

**Distribution of antibody concentrations**

The distribution of indicative antibody levels ( $\geq 1$ ;  $> 0.15$  to  $< 1$ ;  $\leq 0.15$ ) according to age in the 0 to 9 year old age groups is shown in the Figure. It was

observed that both short-term and long-term protection increased with age, indicating natural infection before routine vaccine administration. The antibody levels considered non-protective were 58.0%, 35.3%, 28.1%, 19.8%, 18.4%, 15.3%, and 20.6% among children under 5 years of age, 5-9, 10-19, 20-29, 30-39, 40-49, and 50 and over age groups, respectively. The protection against Hib infection increased with age in all 3 provinces. The protective level ( $\geq 1.0 \mu\text{g/mL}$ ) of Hib antibody in Antalya province (72.1%) was higher than in Diyarbakır (68.3%) and Samsun (62.8%) with a statistically significant difference of  $P = 0.003$ . The distribution of antibody concentrations according to age, gender, and location by protection levels is shown in Table 1. The older age groups had higher levels of antibody concentration, especially the participants over 15 years of age, who demonstrated non-protective levels of antibody in less than 20% of the sera.

Results of the logistic regression analysis are presented in Table 2. The independent variables were age, gender, and location, which might influence the immunity. According to LR analysis, antibody response to Hib increases with age (Antalya: OR 1.028, 95.0% CI 1.070-1.038; Diyarbakır: OR 1.027, 95.0% CI 1.060-1.038; Samsun: OR 1.041, 95.0% CI 1.030-1.052).

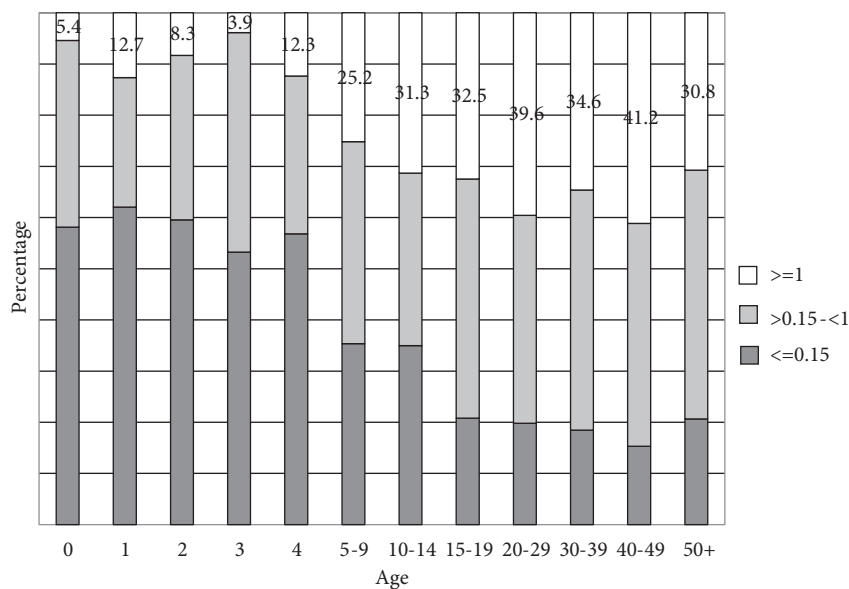


Figure. The distribution of indicative antibody levels ( $\geq 1$ ;  $> 0.15$  to  $< 1$ ;  $\leq 0.15$ ) according to age between the 0 to 9 years old age groups.

Table 1. The distribution of Hib antibody levels according to age, gender, and location in Antalya, Diyarbakır, and Samsun; 2000-2001.

	Antalya				Diyarbakır				Samsun			
	n	≤ 0.15*	> 0.15-< 1**	≥ 1***	n	≤ 0.15	> 0.15-< 1	≥ 1	n	≤ 0.15	> 0.15-< 1	≥ 1
		%	%	%		%	%	%		%	%	%
Age gAAge Group												
0-4	135	50.4	39.3	10.4	113	50.4	41.6	8.0	147	70.7	21.8	7.5
5-9	130	30.0	44.6	25.4	113	37.2	38.1	24.8	122	39.3	35.2	25.4
10-19	52	19.2	48.1	32.7	53	34.0	39.6	26.4	55	30.9	32.7	36.4
20-29	75	18.7	41.3	40.0	60	26.7	30.0	43.3	62	14.5	50.0	35.5
30-39	68	17.6	48.5	33.8	51	17.6	51.0	31.4	60	20.0	41.7	38.3
40-4	64	18.8	50.0	31.3	50	16.0	38.0	46.0	56	10.7	41.1	48.2
50+	85	17.6	48.2	34.1	84	19.0	44.0	36.9	78	25.6	53.8	20.5
Gender												
Male	279	26.5	45.9	27.6	254	34.3	39.0	26.8	283	36.0	40.3	23.7
Female	30	9.1	43.9	27.0	270	29.3	41.5	29.3	297	38.4	33.7	27.9
Location												
Rural	97	7.3	44.4	28.3	287	33.1	36.9	30.0	293	37.9	34.5	27.6
Urban	312	28.5	45.2	26.3	237	30.0	44.3	25.7	287	36.6	39.4	24.0
Total	609	27.9	44.8	27.3	524	31.7	40.3	28.1	580	37.2	36.9	25.9

\*non-protective

\*\*short term protection

\*\*\*long term protection

Table 2. Logistic regression analysis for protective levels (<1µg/mL, ≥1 µg/mL); Antalya, Diyarbakır, and Samsun.

	Antalya				Diyarbakır			Samsun				
	OR	95.0% CI		P	OR	95.0% CI	P	OR	95.0% CI		P	
Age	1.028	1.017	1.038	0.000**	1.027	1.016	1.038	0.000**	1.041	1.030	1.052	0.000**
Gender												
Male*	1				1				1			
Female	0.894	0.620	1.289	0.549	1.205	0.825	1.761	0.335	0.871	0.609	1.246	0.451
Residence												
Rural*	1				1				1			
Urban	0.945	0.657	1.360	0.762	1.141	0.779	1.671	0.499	1.074	0.751	1.536	0.694

\*: Reference group

\*\*: statistically significant

### Discussion

The most persuasive way to demonstrate the effectiveness of a Hib vaccine is to demonstrate a decrease in the incidence of Hib-associated disease. In countries where mass vaccination has been introduced, a reduction of up to 99% in disease incidence has been demonstrated (10,11). In Turkey, Hib vaccine was introduced into the routine childhood immunization schedule in January 2007.

However, the true incidence of Hib-associated diseases in Turkey, either currently or immediately before the introduction of routine immunization, is not known. The laboratory-based infectious diseases notification system in Turkey is currently being improved, but the system is unlikely to improve quickly enough to provide comprehensive disease incidence data in the immediate future. An alternative way to monitor the effectiveness of a vaccine is to

measure the levels of induced antibodies in vaccinated individuals, or through serosurveillance activities. To ensure valid comparisons, baseline data should be available and include the total population.

This study was designated to provide baseline data before routine vaccination implementation. Invasive *Haemophilus influenzae* type b (Hib) infection is associated with significant morbidity and mortality in children under 5 years of age, (10-14) and so we focused on this age group. According to our results, children less than 5 years of age were shown to be susceptible to the infection due to low Hib antibody levels (Figure). In addition, a percentage of both short and long term protection levels increased until 10 years of age, which indicates natural infection in the absence of vaccination, which was also shown by others (1).

Other studies performed in this country had shown natural immunity to Hib between 65.3% and 68%, which is higher than our findings (15,16). However, natural immunity to Hib can be influenced by geographic distribution, as well as genetic and socioeconomic factors (15). Studies carried out in healthy children in Turkey suggest that nasopharyngeal colonization with Hib varies between

2.1% and 47.5%, which may be an important source of transmission in nurseries and kindergartens, especially the individuals who are at this age with low-level antibody as shown by our study (2,7,8).

Others have shown that natural immunity may differ by age, gender, and geographical location, which is also demonstrated in this study where a statistically significant difference was found among the 3 provinces (12). The older age groups (>15 years of age) had higher levels of antibody concentration, with less than 20% showing non-protective levels of antibody. On the other hand, in this study, the natural immunity percentage according to gender was not found to be statistically significant.

This study has provided baseline data that may be used for comparison in future surveillance studies in Turkey, and potentially as comparative data in similar studies in selected neighbouring countries.

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