Short Communication

Comparison of *Haemophilus influenzae* Seroprevalence in Serum Samples Collected from 0- to 5-Year-Old Japanese Children in 1980, 1995, 2010, and 2012

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SUMMARY: *Haemophilus influenzae* type b (Hib) causes several invasive infections such as meningitis, septic arthritis, and pneumonia, especially in children below 5 years old. Despite the availability of Hib vaccines against Hib infection, seroepidemiological surveys of Hib infections have not yet been systematically conducted in Japan. We analyzed 1,338 serum samples, provided by the National Serum Reference Bank of the National Institute of Infectious Diseases (Tokyo, Japan), from 0- to 5-year-old children. Anti-polyribosylribitol phosphate (PRP) immunoglobulin G (IgG) antibody levels against Hib were determined using an anti-*H. influenzae* IgG enzyme immunoassay kit. In a total of 1,168 (87.3%) serum samples from children, anti-PRP IgG antibody levels were $\geq 0.15 \mu\text{g/mL}$, providing natural immunity. Titers expected to provide long-term protection ($\geq 1 \mu\text{g/mL}$) were increased from 2.7% to 51.6% in children < 1 year old after the introduction of Hib vaccine (1980, 5.3%; 1995, 2.7%; 2010, 22%; 2012, 51.6%). Our data confirmed that the introduction of Hib vaccination in children below 5 years old increased the proportion of children having high anti-PRP IgG antibody levels, ensuring long-term protection against Hib.

Haemophilus influenzae type b (Hib) is one of the most important bacterial pathogens that causes several invasive infections with high mortality and morbidity, especially in children below 5 years old (1–3). In Japan, sentinel surveillance for bacterial meningitis has been conducted since 1981. A recent report published in 2010 suggested that recorded cases of bacterial meningitis during sentinel surveys had been gradually increasing from 2006 to 2009 (4). Children below 5 years old comprise the main target population for bacterial meningitis, and the most invasive infections occur in children below 12 months old (1–3). The first Hib-conjugated vaccine was created in the 1970s in the USA (5), and by March 2013, it was licensed for the prevention of invasive meningitis in children in more than 180 countries (6). Hib-conjugated vaccines are highly effective in preventing invasive Hib infectious diseases in children younger than 5 years, especially in children below 2 years old. Although Hib vaccine has been introduced in Japan on a volunteer-based program since December 2008 and for a temporary national project between November 2010 and March 2013, it has not been used routinely before April 2013. Protective immunity induced by Hib vaccine against Hib infection is assessed by measuring the concentration of anti-polysaccharide polyribosylribitol phosphate (PRP) IgG antibody, with levels $\geq 0.15 \mu\text{g/mL}$ and $\geq 1 \mu\text{g/mL}$

providing short- and long-term protection, respectively (7).

The present study aimed to retrospectively examine the seroprevalence of anti-PRP IgG in healthy children below 5 years old to confirm the enhanced immunity to Hib after the introduction of Hib vaccine in Japan.

Serum samples, collected in 1979–1980, 1994–1995, 2010, and 2012 in 25 prefectures across the country, from a total of 1,338 healthy 0–5-year-old children were supplied by the National Serum Reference Bank (SRB) of the National Institute of Infectious Diseases (Tokyo, Japan). The SRB provides serum samples collected from healthy residents across the nation for future seroepidemiological surveys. Anti-PRP IgG antibody levels in human sera were determined using a VaccZyme human anti- Hib enzyme immunoassay kit (Binding Site, Birmingham, UK) according to the manufacturer's instructions.

Anti-PRP antibody levels were determined in serum samples from a total of 1,338 healthy children including 740 samples from males, 596 samples from females, and 2 samples from individuals with unknown sex (Table 1). Analysis of the prevalence of anti-PRP IgG antibody levels showed that in 12.9% of samples collected in 1980, 19.4% of samples collected in 1995, 17.7% of samples collected in 2010, and 17.3% of samples collected in 2012, IgG antibody levels were below $0.15 \mu\text{g/mL}$. The relationship between anti-PRP IgG antibody levels in serum samples, age, and collection year is shown in Fig. 1. High anti-PRP IgG antibody levels ($\geq 5 \mu\text{g/mL}$) (8), which provide long-term protection against Hib infection, were observed in 429 children (a total of 32% of children) (1980, 17.4%; 1995, 17.1%; 2010, 26.3%; 2012, 68%), including 49 children below 1 year old (a total of 18.7% of children < 1 year old) (1980, 5.3%; 1995, 2.7%; 2010, 22%; 2012, 51.6%).

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Fig. 1. Distribution of the levels of the anti-Hib IgG (anti-polysaccharide polyribosylribitol phosphate [PRP]) antibody in serum samples collected from < 1, 1, 2, 3, 4, and 5-year-old children in 1980 (A), 1995 (B), 2010 (C), and 2012 (D) in Japan.

Table 1. Numbers of subjects whose serum samples were collected during 1979 to 2012

Year	Sex	Age (years old)						Total
		0	1	2	3	4	5	
1979	Male		5	3				8
	Female		7	3				10
1980	Male	39	32	26	34	32	29	192
	Female	36	31	18	16	18	21	140
1979 and 1980		75	75	50	50	50	50	350
1994	Male	23						23
	Female	16						16
1995	Male	21	36	31	25	31	28	172
	Female	15	37	19	25	19	22	137
	Unknown		2					2
1994 and 1995		75	75	50	50	50	50	350
2010	Male	25	26	31	29	25	26	162
	Female	25	24	19	21	25	24	138
Total of 2010		50	50	50	50	50	50	300
2012	Male	35	41	27	27	23	30	183
	Female	28	34	23	23	27	20	155
Total of 2012		63	75	50	50	50	50	338
Total		263	275	200	200	200	200	1,338

Natural rates of the samples positive for anti-PRP IgG antibodies have been gradually increasing in children < 1 year old with cumulative age in Japan in 1994–1995 and in 2010. Anti-PRP IgG antibody levels providing natural immunity (> 0.15 µg/mL) were observed in 87.1% and 80.6% of samples collected in 1979–1980 and 1994–1995, respectively. A recent study from Turkey (9) demonstrated the presence of natural immunity against Hib in 53.4%–65.3% of the samples. In our study, the natural immunity rates were high at very early ages even before Hib vaccine was introduced. Therefore, a significant increase in natural immunity level was not observed with age. In serum samples collected from children in 1979–1980 and in 1994–1995, when Hib vaccine was not yet introduced nationwide in Japan, the prevalence of the anti-PRP antibody revealed a gradual, age-dependent increase in children < 1 year old.

Notably, anti-PRP IgG antibody levels greater than 1 µg/mL were observed in 5.3% (8/150) and 2.7% (4/150) of serum samples from children < 1 year old collected in 1979–1980 and 1994–1995, respectively (Fig. 1). Ishiwada et al. reported that anti-PRP titers did not increase after systemic Hib infection in children below 16 months old (10). However, a significant increase in anti-PRP IgG antibody levels during systemic infection was detected in 24-month-old children. In Japan, the vaccine against Hib was introduced as a voluntary vaccine in December 2008. The manufacturer provided approximately 70,000–80,000 doses of vaccine monthly from April 2009 to March 2010. In total, ~970,000 and ~5,500,000 doses were provided between April 2009 and March 2010 and between April 2011 and March 2012, respectively. In 2010, serum samples were collected from April to September, but mainly from July

to September. The high antibody prevalence rates in children below 1 year old in 2010 presumably suggest that a significant number of vaccinated individuals were included in those populations. Unfortunately, our serum samples did not include vaccination histories, which was the main limitation of this study.

In Japan, a number of routine vaccines are already being used nationwide; hence, the introduction of yet another vaccine will interrupt the vaccination schedule. Adjustments to the vaccination schedule by improving inoculation methods, using multivalent vaccines, and performing simultaneous inoculations will be required to optimize the strategy. Additionally, the high-quality nationwide surveillance of the invasive Hib infection will provide reliable information on the effectiveness of the vaccination strategy. Therefore, periodical sero-surveys and high-quality routine patient and pathogen surveillance reports should be kept for planning national comprehensive multiyear strategic plans for the immunization in Japan.

In the USA, the number of Hib meningitis cases in children below 5 years old was 24 per 100,000 population in 1984. Since the introduction of Hib vaccine, the incidence was reduced to 3.7 per 100,000 population in 1991 (11). In Japan, Suga et al. reported that the incidence of bacterial meningitis caused by Hib infection decreased to 92% after the introduction of Hib vaccine in children below 5 years old (2008–2010, 7.7/100,000 population; 2012, 0.6/100,000 population) (1). These findings were consistent with the increased proportions of children below 5 years old with anti-PRP IgG concentration providing long-term protection (≥ 1 µg/mL). The authors recently reported that the incidence of invasive *H. influenzae* diseases in children below 5

years old dramatically decreased from 6.2 in 2011 to 0.9 per 100,000 population in 2017 (12). The proportion of Hib, the causative organism of invasive disease, among all *H. influenzae* strains was 88.9% in 2008–2012, but decreased to only 3.2% in 2013–2017, indicating a dramatic decrease of Hib infection in children. However, recently, non-Hib infection rate in children below 5 years old has increased in Japan (12). Similar case has been observed in many countries that introduced Hib vaccine against Hib infection in children below 5 years old. Accurate data regarding serotype replacement of *H. influenzae* infection in Japan has not been demonstrated yet. We believe that accurate nationwide surveillance is helpful in determining future epidemiological changes in the rate of invasive *H. influenzae* infection in Japan.

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Conflict of interest None to declare.

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