Short Communication

The prevalence of natural immunity to Hemophilus influenzae type b in Iranian children

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DOI: 10.22034/HBB.2019.17
Received: July 3, 2019; Accepted: July 29, 2019

ABSTRACT

Haemophilus influenzae causes various infectious diseases such as meningitis, septic arthritis, bacteriemia, ampyeme and cellulites. Although all subtypes of H. influenzae cause these infectious conditions, H. influenzae type b (Hib) is the most common subtype with an incidence of 95% and it is known to be a major cause of mortality and morbidity among children under 5 years of age in Asia. Hib vaccine is not entered in routine immunization program in children in Iran yet. The present results show that most Iranian infants that are not vaccinated with Hib-conjugate vaccines are susceptible to Hib diseases, which confirm the previous findings. We found that the protective titer of antibody against Hib is very low in children under 1 year old and gradually increases with age as it shown in other study. The decrease in the prevalence of Hib-associated disease in older children appears to be associated with age-related increases in anti- capsular polysaccharide (anti-CP) titers.

Keywords: H. influenzae type b, Iranian infants, immunity

INTRODUCTION

Haemophilus influenzae causes various infections disease such as meningitis, septic arthritis, bacteriemia, ampyeme and cellulites. Although all subtypes of H. influenzae cause these infectious conditions, H. influenzae type b (Hib) is the most...
common subtype with a frequency of 95% and it is thought by some authorities to be insignificant in Asia [1-3], either because of true biologic or geographic variation in the incidence of invasive Hib disease, perhaps because of failure to detect invasive disease [4,5]. Eighty-five percent of H. influenzae cases happen in children less than 5 years of age. The incidence of Hib in infants under 6 months age is lower as a result of the antibodies achieved from placenta and breastfeeding [6,7].

It was shown that the incidence of Hib disease on children between 0 and 5 years of age in developed countries is 20-100/100 000 and consequently, it was suggested that the conjugated Hib vaccine would be applied normally [8,9]. The protective effect of the vaccine is more than 90% [2,10,11]. In developing countries the incidence varies between 10 and 77/100,000 [12,13]. On the base an Iranian study the incidence of Hib infection was estimated 43.0 per 100000, which can be reduced to 6.7 by vaccination [14].

The World Health Organization (WHO) has suggested that surveillance for Hib disease needs to be undertaken in less developed countries to determine the prevalence of Hib disease and to ascertain the potential utility of Hib vaccines in those regions [2,15]. Even if the assessment by the WHO is that aggressive Hib diseases account for about 400,000 deaths of children under 6 years of age each year worldwide, Hib conjugate vaccines are not still commonly used in developing countries, Hib vaccine is not also entered in routine immunization programs for children in Iran up to date [16].

In newborn, maternal anti-PRP (Poly-Ribitol Phosphate) IgG provides protection; but, when maternal antibody in the infant declines, the risk for infection and present of Hib disease increase. As children approach 2 years of age, exposure to the organism cause to appear their own antibody to the capsular polysaccharide begins to appear. The antigenic stimulus for this age dependent development of bactericidal activity may be through mucosal exposure to Hib or to other cross reacting antigens like Escherichia coli K 100 [17-21].

The introduction of Hib conjugate vaccine into routine vaccination in industrialized countries has virtually eliminated invasive Hib disease in children aged 0-5 years [22,23]. The antibody response of Hib conjugate vaccine produces immunologic memory and long-term protection against Hib infection.

There is still no study in Iran that gives the overall incidence of Hib disease. The aim of
the present study was to determine the levels of Hib antibodies in non-vaccinated Iranian young children.

Venous blood specimens were drawn from 1086 infants and young children aged 2 months to 6 years who visited Imam Husain, Milad or Baharlou Hospital on three different geographic zones in Tehran, Iran during Feb 2010 until Feb 2011. The majority of these children visited the hospital for normal visits. None of the cases had previously suffered from systemic Hib infection, received Hib vaccine, or received any immunoglobulin. After blood-clotting at room temperature, all tubes were centrifuged. Sera were collected and stored at −80°C until the day of analysis. The study was approved by the Ethics Committee of Pasteur Institute of Iran (Tehran).

The ELISA procedure has several benefits compare to other methods because assay of large numbers of samples will be done without using of radioisotopes [28,29].

Anti-CP antibody titers were analyzed on a Bindazyme Anti-Haemophilus b Enzyme Immunoassay Kit (Binding Site, Birmingham, UK Code: MK016). The test is based on the addition of the serum samples in a proper manner to the microtitration plates coated with the PRP (Poly-Ribitol Phosphate) antigens, and scanning of the color change obtained as a result of various processing by the optic intensity scanner, and finally determination of the antibody values according to the standard curves drawn. Each kit was examined on the day of opening. The lowest and highest limits of detection for anti-CP IgG antibody were 0.01 and 9 μg/ml, respectively. Antibody levels more than 0.15 μg/ml were accepted as having good inverse correlation with the incidence of disease [26]. When the anti-CP antibody titer was more than 9 μg/ml, the sample was diluted and reexamined.

The $p$ value is measured by using t-test.

A total of 1086 children (57% male and 43% female) were included in the present study. 96% were breast-fed exclusively for more than 10 months (Table 1).

There was no significant positive correlation between Hib positivity (Anti-CP antibody level more than 0.15 μg/mL is considered to be protective threshold at the time of exposure) and gender, in breast feeding children exclusive breast-feeding duration ($p>0.05$).

Anti-CP antibody titers of 0.15 μg/ml were accepted as the minimum level required for protection. In the present study 561 children (51.5%) did not have the minimum level required for protection against Hib. Titers
expected to be protective for immediate but short-term periods (0.15-0.99 µg/ml) were observed in 253 children (23.3 %) or have protective level of antibody for urgent protective but short-term periods. Sixteen percent of children had long-term protective anti-CP antibody titers of 1-5 µg/ml, and only 9% had titers more than 5 µg/ml. To analyze the prevalence of anti-CP antibodies, we divided the children into three groups by age: 2-24 months (n=371), 25-48 months (n=358) and 49-72 months (n=357). We found the lowest levels of anti-CP antibody in children under 12 months old (Table 1). Thirty eight percent of < 24 months children show the protective level of anti-CP antibody titers (Table 1). In the other age groups we have seen the protective level of anti-CP antibody in 45.3 % of 2-4 and 61.4 % of 4-6 years old. A fascinating finding in the present study was the existence of natural immunity in 50 % of non-vaccinated young children under 5 years of age, almost similar to the frequency obtained in other studies, although, Sotoodeh Jahromi et al reported about 85 % natural immunity in South of Iran (Jahrom) as a local area [34]. Ocaktan et al. reported the presence of protective anti-CP antibodies in 64 % of 13 months old infants [6]. The present study provides strong evidence that children in some developing countries acquire natural immunity to Hib at an early age but it is necessary to administer Hib vaccine to all children under 5 years old. Another study performed in West Africa and France reported that there was no detectable natural Hib antibodies in children older than 4 years of age [21]. As declared before Hib conjugate vaccine is the only way to produce protective levels of anti-CP antibody titers against Hib infections in young children [29]. Kaythy et al. reported that 79 % of children acquired anti-CP titers higher than 0.15 µg/ml and 32 % titers greater than 1 µg/ml
after vaccination [37]. These studies, which performed in children population with a high prevalence of susceptible subjects, are in accordance with numerous studies carried out in the USA and Finland on the distribution of the Hib pathology. These reports confirmed the high incidence of infections caused by this micro-organism in the first years of life in unvaccinated children [38].

Before the wide spread use of conjugated vaccines, Hib colonized the nasopharynx of children at a rate of 2% to 4% under 5 years of age [39]. It is identified from our results that 48.5% of Iranian children have protective level (≥0.15 µg/ml) of PRP antibody (Table 1), this rate is so more than other investigations reported [39].

Unfortunately this study was not designed to evaluate the colonization of Hib or the rectal carriage of *Escherichia coli* K 100, an organism with a capsule antigenically similar to Hib[17].

As we can see in references the level of maternally acquired serum antibody to PRP declines after birth and reaches a nadir at approximately 18 to 24 months of age, the peak age incidence of meningitis caused by

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Hib in an unimmunized child. The level of antibody to PRP then gradually rises, apparently as a result of exposure to *Haemophilus influenzae* type b or cross-reacting antigens [39], it has been seen in our results, respectively.

### CONCLUSION

In comparison of our results with other studies, it is obviously identified that a large number of Iranian children are at risk of this mortal bacteria [8].

We do not have any idea about the source of the high anti-CP antibody in 48.5% of participants that have high level of antibody. This may be a cause of invasive disease or asymptotic one, but it is obviously a sign of exposure to *Haemophilus influenzae* type b (Hib). These reports confirmed results support the recommendation of the WHO that the strategy of administering Hib vaccine to all children under 5 years old must be considered in all countries [40].

Under the present conditions in Iran, it is strongly expected to inject Hib vaccine for young infants (<2 years of age).
Table 1. Serum anti-CP antibody titers measured by ELISA* in Iranian (Tehran) children Anti-CP antibody

| Anti-CP** IgG antibody titer (µg/ml) | Age (months) | | | |
| | 2-24 | 25-48 | 49-72 | Total |
| | No. | % | No. | % | No. | % | No. | % |
| <0.15 | 228 | 61.4 | 196 | 54.7 | 137 | 38.6 | 561 | 51.5 |
| 0.15-0.99 | 46 | 12.3 | 96 | 26.6 | 111 | 30.9 | 253 | 23.3 |
| 1-5 | 74 | 20.1 | 39 | 11.1 | 64 | 18 | 177 | 16.2 |
| >5 | 23 | 6.2 | 27 | 7.6 | 45 | 12.5 | 95 | 9 |

*ELISA, enzyme-linked immunosorbent assay

** Anti-CP, anti-capsular polysaccharide-specific IgG

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