

Protection against *Haemophilus influenzae* Type B, Diphtheria, and Tetanus in Children from Major Cities of Pakistan: A Community-Based Study

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Abstract

Objective Pentavalent vaccine has been introduced in Pakistan against tetanus, diphtheria, *Haemophilus influenzae* type B (HiB), pertussis, and hepatitis B virus.

Methods This study was designed to determine protection against diphtheria, tetanus, and HiB in children of age 18 to 24 months from community which had received all three doses of pentavalent vaccine.

Results Overall, 97% were having immunity against HiB, 97% against tetanus, and 88% against diphtheria. Our study showed that children aged 18 to 24 months in Pakistan have high level of protection against HiB and tetanus. However, the protection for diphtheria was low in 40% of children which could be improved by adding a booster dose of diphtheria.

Conclusion By increasing the vaccine coverage among children, the burden of these diseases can be reduced in the future.

Keywords

- ▶ pentavalent vaccine
- ▶ vaccine efficacy
- ▶ vaccine coverage
- ▶ Pakistan

Introduction

Pakistan is a country with very high infant's mortality and morbidity. According to the World Health Organization, 393,959 children died in 2013.¹ Nearly half of childhood deaths are among children younger than 5 years, compared with 8 to 10% of deaths in developed countries² mainly because of unhygienic life style, unawareness about the infectious diseases, and poor health facilities. In Pakistan, funds allocation for health are very low³ and per capita

income is negligible.⁴ Therefore, it is difficult for the individuals to avail good health facilities.

The major proportion of children deaths (around one-third) in Pakistan is due to vaccine preventable infectious diseases.¹ To improve the health care system and ensure a better management of the infectious diseases, Pakistan government's expanded program on immunization (EPI) introduced the pentavalent vaccine in 2009,⁵ which is provided free of cost by the government. The pentavalent vaccine has simplified the immunization activities by

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decreasing pain and the number of injections. The combination of vaccines has helped in getting the incorporation of other vaccines in the immunization schedule.⁶ The pentavalent vaccine combines five vaccines in one injection to protect against *Haemophilus influenzae* type B (HiB), diphtheria, pertussis (whooping cough), tetanus (DPT), and hepatitis B.⁵ It has replaced the previous DPT and hepatitis B vaccine. Three doses of this vaccine are given to children in Pakistan during the first year, at 6, 10, and 14 weeks of age.^{2,5}

Diphtheria is a life-threatening disease. *Corynebacterium diphtheriae*, a toxigenic strain, is responsible for this disease.⁷ The second species in this genus *Corynebacterium ulcerans* is also thought to be responsible for the cause of cutaneous diphtheria with the symptoms of sepsis after touching domestic cats, dogs, and foxes.⁸ The mode of transmission of diphtheria is through physical and respiratory contact. Diphtheria may lead to difficulties in breathing and cause death.⁷ In Pakistan, 45 cases of diphtheria have been reported in 2014 of which 30 cases from Punjab, 11 cases from Khyber Pakhtunkhwa (KPK), 3 cases from Sindh, and 1 case from Azad Jammu and Kashmir.⁹

The bacteria which are present in soil named *Clostridium tetani* are the cause of tetanus. The spasms and muscular stiffness are the typical symptoms of tetanus that are caused by tetanus which blocks the inhibitory neurotransmitter in the central nervous system. It is not transmitted from one individual to another. When dirt particles enter a wound or cut, then the individual becomes infected.¹⁰ Pakistan is among one of the 24 countries across the globe where maternal and neonatal tetanus is not still eliminated. Recently, it has been reported that 60 districts of Pakistan out of 139 are at high risk for tetanus infection.¹¹

HiB, a gram-negative bacterium, is a pathogen causing a serious disease among children all over the world. Its frequent symptoms are pneumonia and meningitis.^{12,13} HiB is also responsible for the infections of soft tissues, epiglottis, bones, and joints.¹⁴ A recent report highlights the prevalence of HiB in Pakistan. Children under the age of 5 years were investigated and found that 45 children out of 1,481 were having HiB infections.¹⁵ A meta-analysis of 18 studies comparing the immunogenicity of combined vaccines versus separate vaccines showed that there was a significantly less immunological response for HiB and hepatitis B virus.¹⁶

Immunization is an established cost-effective strategy and public health intervention to control and eradicate the infectious diseases especially in children. Pentavalent vaccines are a combination of five vaccines against DPT, hepatitis B, and HiB. It was added in routine EPI of Pakistan in 2009. A need was felt to determine the protection level of children against these deadly diseases in our local setting as there was no information available in the literature from Pakistan at national level. The present study describes the findings of serosurvey conducted in all major cities of Pakistan to determine the protection against tetanus, HiB, and diphtheria in children.

Materials and Methods

This was a community-based multicenter study which was conducted in four major cities (Rawalpindi, Karachi, Peshawar,

and Quetta of four provinces, i.e., Punjab, Sindh, KPK, and Balochistan, respectively) of Pakistan.

The study protocols and informed consent documents were approved by the Institutional Bioethics Review Committee of the Pakistan Health Research Council (PHRC). Informed consent for the infants was obtained from their parents or legal guardians prior to enrolling the infants in the study.

The sample size was calculated using the coverage rate reported by EPI in different regions of Pakistan. EPI has claimed 20 to 80% coverage in different regions of Pakistan. Assuming a 50% coverage rate and by taking 5% margin error and 95% confidence interval, the sample size of 384 was calculated for each site. However, it was done in two cities, that is, Karachi (Sindh) and Peshawar (KPK) where 384 children were enrolled, while in Rawalpindi (Punjab) and Quetta (Balochistan), 374 and 212 children were enrolled, respectively; therefore, total sample came out to be 1,354.

Participants were selected using a multistage cluster sampling technique. Every selected city was divided into four clusters. Using a lottery method out of four, one cluster was selected. In the selected cluster, lists of households were obtained and starting from the periphery, every second house was surveyed. In case of lock or nonresponse, the next household was visited.

Healthy infants of age 18 to 24 months who had received all three doses of pentavalent vaccine were included, whereas those with recent/past history of acute or chronic illness were excluded.

After selection of the houses and taking the informed written consent from parents/guardians, a pretested questionnaire was filled which included information related to demography and vaccination. About 3 mL blood sample was drawn from the enrolled infants. Blood was centrifuged at 3,000 rpm for 5 minutes and sera were separated. About 500 μ L of the serum was stored at -20°C till analyzed.

Samples were tested for the presence of tetanus, HiB, and diphtheria antibodies using enzyme-linked immunosorbent assay (ELISA) at PHRC Research Centers.

For determination of immunoglobulin G (IgG) antibody levels for diphtheria, certified kits from DeMeditec, Germany were used having sensitivity and specificity of 94%. The IgG antibody cutoff value considered for protection was ≥ 0.1 IU/mL for diphtheria.

For determination of IgG antibody levels for HiB, human HiB IgG ELISA kit of Creative Diagnostics, United States was used having intra-assay variation $\leq 10\%$ and interassay variation 9 to 12%. The antibody concentration < 0.15 $\mu\text{g/mL}$, 0.15 showed insufficient protection against HiB. However, the antibody concentration between 0.15 to 1.0 and > 1.0 $\mu\text{g/mL}$ indicated immunization with polyribosyl ribitol phosphate (PRP) and sufficient natural immunity (SNI) or an acquired protection after the third vaccination, respectively.

For determination of IgG antibody levels for tetanus, certified kits from Vircell, Spain were used with 96% sensitivity and specificity. Samples with indexes above 11 were considered as having IgG-specific antibodies against tetanus. The index was calculated as (sample optical density [OD]/

cutoff serum mean OD) $\times 10$. Positive, negative, and cutoff controls were provided with the kit.

Standards and test serum samples were tested in duplicate. The absorbance was measured with an ELISA reader at 450 nm. The mean OD values were calculated from two readings of each sample. The validation criteria mentioned in kit literature was strictly adhered to. From the OD values of standards, a reference curve was plotted, and finally, the concentration of IgG antibody was determined for each sample. The serological testing was performed at the PHRC Research Centers of Peshawar and Karachi following Good Laboratory Practice guidelines.

Results

A total of 1,294 children were enrolled including 350 from Peshawar and Karachi each, while 348 from Rawalpindi and 212 from Quetta. Among all, 771 (57%) were males and 583 (43%) were females.

For HiB, overall 97% were immune against the disease and among them, 56% were had SNI, while 41 showed antibodies against vaccine, that is, immunized with polyribosyl ribitol phosphate (IPRP). The remaining 3% did not have either SNI or antibodies against IPRP making them vulnerable to disease.

Within the cities, the children belonging to Quetta city (Balochistan) had highest protection, that is, 96% (SNI) against HiB followed by 61% in Karachi (Sindh), 41% in Peshawar (KPK) and Rawalpindi (Punjab) each. Majority (57%) were IPRP in Peshawar followed by 51% in Rawalpindi, 38% in Karachi, and 41.1% in Quetta. No age-specific difference regarding HiB has been observed in this study (**►Tables 1–3**).

Overall 96% were positive for tetanus, and the rests were negative and/or equivocal. When compared between cities, 99% were positive in Peshawar, 98% in Quetta, and 97% in Rawalpindi followed by 92% in Karachi. Almost 6% were found to be not protected in Karachi which is higher as compared with other cities. There was no significant variation with the age (**►Tables 1–3**).

Regarding diphtheria, overall 63% were fully protected, 25% were somewhat protected, while 12% were not pro-

Table 2 *Haemophilus influenzae* type B within 18 to 24 months age group

Age (mo)	Number	SNI	IPRP	ISNI	TP	%
18	268	162	99	4	261	97
19	94	62	31	1	93	99
20	138	86	47	5	133	96
21	71	52	18	1	70	99
22	81	53	27	1	80	99

Abbreviations: IPRP, immunized with polyribosyl ribitol phosphate; ISNI, insufficient natural immunity; SNI, sufficient natural immunity; TP, total (overall) protection.

tected. In the case of Quetta, the majority (92%) were fully protected followed by almost 62, 57, and 56% in Karachi, Rawalpindi, and Peshawar, respectively. About 36% were somewhat protected against diphtheria in Karachi followed by 19.3% in Rawalpindi. Almost 23% in Peshawar and 21% in Rawalpindi were not protected against diphtheria. Also, the protection level showed a downward trend with an increase in age, but this was not consistent (**►Table 4**).

Discussion

The dire need of the day is the prevention of such diseases which are preventable by vaccines. It has been observed that every year approximately three millions of children die globally from vaccine preventable diseases.¹⁷ Over the past two decades, DPT vaccination coverage has been improved in Pakistan. The DPT trivalent vaccination coverage was 35% in 1990–1991, then it has been increased to 59% in 2006–2007.¹⁸ The recent Pakistan demographic health survey reported that coverage of three doses of trivalent vaccine was 65% in 2012–2013. The information was taken from both vaccination cards and mother sources. The proportion of coverage, however, varies within cities. The coverage of three doses of trivalent vaccine was 76% in Punjab followed by 69.6% in KPK, 38.6% in Sindh, and 27% in Balochistan. More male children were vaccinated than female children.¹⁸ The

Table 1 HiB, tetanus, and diphtheria in different cities of Pakistan

Province/city	HiB			Tetanus			Diphtheria		
	SNI N (%)	IPRP N (%)	ISNI N (%)	Positive N (%)	Negative N (%)	Equivocal N (%)	FP N (%)	SP N (%)	NP N (%)
Punjab/Rawalpindi	141 (40.5%)	178 (51.1%)	29 (8.3%)	340 (97.7%)	2 (0.6%)	6 (1.7%)	208 (59.8%)	67 (19.3%)	73 (21%)
Sindh/Karachi	236 (61.5%)	146 (38%)	2 (0.5%)	352 (91.7%)	23 (6%)	9 (2.3%)	237 (61.7%)	139 (36.2%)	8 (2.1%)
KPK/Peshawar	143 (41%)	200 (57%)	7 (2%)	348 (99%)	2 (1%)	0	209 (60%)	58 (17%)	83 (23%)
Balochistan/Quetta	204 (96%)	532 (41.1%)	38 (2.9%)	207 (98%)	5 (2%)	0	194 (92%)	17 (8%)	1 (0%)

Abbreviations: FP, fully protected; HiB, *Haemophilus influenzae* type B; IPRP, immunized with polyribosyl ribitol phosphate; ISNI, insufficient natural immunity; KPK, Khyber Pakhtunkhwa; NP, not protected; SNI, sufficient natural immunity; SP, somewhat protected.

Table 3 Tetanus in 18 to 24 months age group

Age (mo)	Number	Positive	Negative	Equivocal	TP	%
18	268	260	4	4	264	99
19	94	91	3	0	91	97
20	138	135	3	0	135	98
21	71	70	1	0	70	99
22	81	78	1	2	80	99
23	106	104	2	0	104	98
24	536	510	17	9	519	97

Abbreviation: TP, total (overall) protection.

Table 4 Diphtheria in 18 to 24 months age group

Age (mo)	Kids	FP	SP	NP	TP	%
18	268	192	52	24	244	91
19	94	72	18	4	90	96
20	138	96	21	21	117	85
21	71	54	8	9	62	87
22	81	58	15	9	73	90
23	106	69	21	16	90	85
24	536	307	151	78	458	85

Abbreviation: FP, fully protected; NP, not protected; SP, somewhat protected; TP, total (overall) protection.

pentavalent vaccine was introduced in Pakistan in 2009, and currently, there is no data regarding the efficacy of the pentavalent vaccine. So, this study was performed nationwide and the results of pentavalent vaccine immunization against tetanus, diphtheria, and HiB are 97, 88, and 97%, respectively.

Various combination vaccines are in practice globally to prevent DPT.¹⁹ Vaccination against tetanus has depicted a major decline in the case of this fatal disease.²⁰ By conducting programs of effective immunization and good postexposure prophylaxis, tetanus is rare in developed countries.^{21,22} A recent report from Poland regarding the immunization status against tetanus in vaccinated children (number = 324; aged 18–180 months) showed that 29% of children were protected, 5.86% were unprotected, and 65.12% were considered as an intermediate.²³ A study from United Arab Emirates regarding the tetanus vaccine efficacy in a group of 227 children showed that 89.9% of children were protected, 4% showed lack of protection, and 2.2% were equivocal against tetanus.²⁴ From Gaza, Al Aswad reported that the protection of tetanus from the vaccine was 95% out of 180 children.²⁵ Our results regarding the immunization status against tetanus by using the pentavalent vaccine showed that regarding tetanus, 99% were protected against this disease in Punjab and KPK, 98% in Balochistan, and 94% in Sindh. Similar findings were also reported from Iran (96%) and Gaza (98%).^{25–27} The exact incidence of tetanus across the globe is unknown and it is estimated that only 10% of cases are reported.²⁸ In 2013, seven cases of tetanus were reported in

the United Kingdom, but it is more common in other parts of the world.²⁹

A significant decline has been observed in the case of diphtheria due to vaccination. Before the introduction of routine vaccination, ~1,800 deaths were recorded related to diphtheria each year.³⁰ Diphtheria antibodies (IgG) can be induced by natural toxin during clinical or subclinical infection and by immunization.^{31,32} In this study, ~63% were fully protected against diphtheria. A similar finding was reported by studies conducted in Islamabad and Israel where fully protected children against diphtheria were 74%.^{33,34} The results showed that the proportion of “nonprotected” children was highest in Punjab and the same were the findings of a study from India where seroprotection was also around 50%.³⁴ A similar finding was reported recently from Poland where the seroprotection against diphtheria was only 29.32%.²³ Our findings were however contrary to those reported from Iran, United Arab Emirates, and Palestine where seroprotection rate against diphtheria was 99, 86, and 87%, respectively.^{24,26,27}

Since the early 1990s, there has been a gradual introduction of the HiB conjugate vaccine into more and more routine programs. In 2004, 89 countries used the vaccine, but in 2011, the number of countries has increased up to 172.³⁵ Before the introduction of vaccines, there was a large number of cases of HiB in different regions and 20,000 cases were estimated annually from the United States.³⁶ The efficacy of the HiB vaccine should also be under observation along with the progress of vaccination. Different reports are available on

the efficacy of HiB vaccine from different countries. The results of this study showed that overall 97% of children were protected against HiB which is similar to the findings of the studies reported from Salvador, India, and England.^{36–38}

Conclusion

There are several diseases that have been eradicated globally by the introduction of vaccines. Although the vaccination preparation and scheduling of vaccines advanced, several vaccine preventable diseases still exist. In Pakistan, the infant's death rate is still higher than the other developing countries. From the past two decades, the vaccination coverage has been improved in Pakistan, which results in decreased infant death rate. The current study showed high level of protection against tetanus and HiB in children aged 18 to 24 months. However, the protection against diphtheria was low in 40% children; therefore, a booster dose for diphtheria at 18 months needs to be added in routine EPI. This will eventually improve the infant's immunization and will reduce the mortality and morbidity against vaccine preventable diseases.

Note

The study protocols and informed consent documents were approved by the Institutional Bioethics Review Committee (IBRC) of Pakistan Health Research Council (PHRC). For infants, informed consent to participate in the study was obtained from their parents or legal guardians prior to enrolling the infants in the study. Informed written consent for publication was obtained from each participant which stated that the details/images/videos will be freely available on the internet and may be seen by the general public.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions

M.A.N.S. and I.R. designed the study. O.U., R.I., N.J., A.K., and R.F. collected the data. M.A.N.S., I.R., A.R., and M.S.A. did the data analysis and wrote the manuscript.

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Conflict of Interest

None declared.

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