IgG SPECIFIC LEVELS FOR DIPHTHERIA, TETANUS AND PERTUSSIS IN RURAL AND URBAN SAMPLES IN ALBANIA

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Abstract

INTRODUCTION: During the years 1970s and 1980s, IPH (Institute of Public Health) in Albania, achieved remarkable improvements in the biotechnology of the components production of D (diphtheria), T (tetanus) and P (pertussis) for the trivalent vaccine DTP, vaccine DT and the Td one, which together with vaccine TT, consequently led to the increasing of their effectiveness and efficiency. In the year 2000, Rubella component was added to Measles vaccine, and in the year 2005, Mumps component was added too (now MMR vaccine).

OBJECTIVE: The primary objective of this study was to identify protective immunoglobulin G (IgG) levels for each antigen (diphtheria, tetanus, and pertussis) according to different age groups and residence (rural and urban).

METHODS: Two samples, each involving 120 healthy children were selected from urban and rural populations of three central regions of Albania. The children were aged between 1 and 15 years. IgG specific levels for diphtheria, tetanus, and pertussis were measured using enzyme-linked immunosorbent assay tests (ELISA). Levels of IgG were classified into the following categories: unprotected, insufficient, and fair/good or full/sure protection, using specific thresholds for each, diphtheria, tetanus, and pertussis.

RESULTS: Of the same population, 75.7% was found to have IgG protective levels for diphtheria. For tetanus, 81.4% of the total sample had protective levels of IgG, and for pertussis this proportion was 69.0%.Levels of protection for diphtheria and tetanus increased up until 3 years of age (diphtheria: 25.0% at 1 year and then 95.0% at 3 years of age; tetanus: 65.0% at 1 year and 90.0% at 3 years of age). From then on the trend stabilized. The same trend was not observed for pertussis, which presented protective IgG levels of 73.0% from the first year of life. When IgG protective levels were compared between rural and urban samples, differences were only found with diphtheria specific IgG, which was significantly higher in the rural population. Furthermore, a correlation in IgG levels was found among diphtheria and tetanus.

CONCLUSIONS: IgG specific levels for diphtheria, tetanus and pertussis referring to our sample are are considered acceptable for providing protection in the general population. Nevertheless, these levels seem to have been achieved only after booster doses until the third year of life, especially for diphtheria.

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Introduction

According to the Center for Disease Control and Prevention (CDC) (2015), tetanus toxoid was developed for the first time by Descombey in 1924 and it was widely used during World War II. II. Tetanus toxoid is available combined with diphtheria toxoid as pediatric diphtheria-tetanus toxoid (DT) or adult tetanus-diphtheria (Td), and with both diphtheria toxoid and acellular pertussis vaccine as DTaP or Tdap (CDC, 2015).

With regard to pertussis, there are no antibodies found specifically that show immunity to an infection of *Bortadella Bordetella pertussis*, according to Cherry (2007). Nevertheless, antibody prevalence at different ages can be an index of exposure to pertussis antigens. After the first infection, an increase of immunoglobulin A (IgA) and IgG against pertussis toxin is noticeable. Campell, Mycntyre, Quinn,

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Hueston, Gilbert and Mc Vernon (2012), found that pertussis re-infection is characterized by a rapid growth of antibodies and this makes diagnosis difficult to determine.

Referring to the literature, the Center for Disease Control and Prevention (1991) stated that the minimum level of tetanus antitoxin correlating with protection is 0.01 IU/ml, as measured by in vivo neutralization assay. An antitoxin concentration of ≥ 0.1 IU/ml is preferred and correlates with protection, based on results of other assays, e.g., enzyme-linked immunosorbent assay (ELISA; Virella & Hyman, 1991), and because higher concentrations of antitoxin might be necessary to provide protection in certain circumstances. The serum level of tetanus antitoxin achieved in response to vaccination is determined by four factors: (1) the number of doses of tetanus toxoid; (2) the type of tetanus toxoid administered (adjuvanted toxoid being more immunogenic, has replaced fluid toxoid); (3) the interval since the most recent dose; and (4) individual variation in the response to vaccination.

Protection against respiratory diphtheria is predominantly a result of IgG antibodies to the diphtheria toxin (antitoxin) induced after infection with toxin-producing *Corynebacterium Corynebacterium diphtheriae* or after vaccination with diphtheria toxoid. Adult populations are becoming more susceptible to diphtheria because repeated doses of diphtheria toxoid are needed to maintain immunity in these populations. As Galazka and Robertson (1996) writes, once a child is immunized, the immunity wanes relatively rapidly without exposure to natural *C. diphtheriae*. Although the immune system responses to infection and vaccination vary, antitoxin concentrations of ≥0.10 IU/ml, according to *Reder*, Riffelmann, Becker and Konig *(2008)*, are typically considered protective. Concentrations of 0.01 IU/ml – 0.10 IU/ml might provide protection against severe disease; referring to Eslamifar, Ramizani, Banifazl, Sofian, Mahdaviani Yaghmaie and Aghakhani (2014), concentrations of <0.01 IU/ml fail to protect against diphtheria, while concentrations of 0.0–0.9 IU/ml provide protection against pertussis. The objective of this study is to identify protective IgG levels for each antigen (diphtheria, tetanus, and pertussis) according to different age groups and place of residence (rural and urban).

Materials and Methodology

Two samples, each involving 120 healthy children, were selected from urban and rural populations of three central regions (Elbasan, Durrës dhe Tiranë) of Albania. The children were aged 1 to 15 years and were grouped by ages: 1 year, 3 years, 5 years, and 15 years.

The IgG specific levels for diphtheria, tetanus, and pertussis were measured using ELISA tests. Levels of IgG were classified as follows: ‘unprotected’, ‘insufficient’, ‘good/ full’ protection using specific thresholds each for diphtheria, tetanus, and pertussis.

The IgG levels for diphtheria classified as having an IgG value < 0.1 IU/ml constituted the category of unprotected. IgG values from 0.10 to 1.40 IU/ mlmL constituted the category of insufficient protection, while respectively, the values from 1.5 to 2.0 IU/ml and > 2.0 IU/ml were regarded “good” and provided optimal protection.For tetanus, <0.10 IU/ml of IgG defined the category of the vulnerable groups; from 0.10 to 1.0 IU/ml insufficient protection; 1.10 to 5.0 IU/ml good protection; and > 5.0 IU/ml optimal protection.

For pertussis, the category with insufficient protection included the level <14.0 IU/ml of IgG, good protection ranged from 14.0 to 18.0 IU/ml, and optimal was > 18.0 IU/ml.

For data analysis, Chi square (X^2 test) was used.

Results and Discussion

Table 1, shows the distribution of the IgG levels for diphtheria by age, which indicate that 75.0% of 1-year-old children present insufficient protection, while immediately after their re-vaccination at the age of 2 the protection levels are increased and specially it shows that children aged 3-years and 5-years had significant greater protection levels.

There appeared a growing tendency in diphtheria protection levels with maximum levels observed in children aged 5 years, followed by a non-significant decline in children aged 15 years. A statistically significant difference was observed for diphtheria protection levels, according to children's age (χ^2= 171 p<0.01).

Table 1: Distribution of protection levels according to age for diphtheria

Age (years)	Protection level according to age for diphtheria (%)
1	25.0
3	95.0
5	100.0
15	90.0
Total protection level for these age groups	75.7

Source: Authors

In the group of children with sufficient protection, 50.0% were aged 5-years, 25.0% 3-years, 16.7% 1-year, and 8.3% 15-years. In the group of children with fair protection, 50.0% were aged 5-years, 36.4% 3-years, 9.1% 15-years, and 4.5% 1-year. In the group of children with optimal protection, 42.1% were aged 3-years, 31.6% 15-years, 15.8% 5-years, and 10.5% 1-year.

Table 2: Distribution of cases according to protection levels and residence for diphtheria

Diphtheria IgG (IU/ml)	Rural Residence children (n=120)	Urban Residence children (n=120)
< 0.10	3	6
0.10 - 0.90	18	27
1.0 - 1.40	15	24
1.50- 2.0	39	33
> 2.0	45	30

Source: Authors

Table 2 shows the IgG levels according to the subjects' residence. The figures seem to show a distinction between rural and urban children. However, most children were within the levels defined as protective or optimal. There were 39 rural children with protective IgG values and 33 urban children with the same level of protection. Only 3 rural children and 6 urban children displayed insufficient protective values for diphtheria. No statistically significant differences were observed for diphtheria protection levels, according to their residence: $\chi2 = 8.3$, $p = 0.07$.

Table 3 shows the distribution of protection level for tetanus according to age. It shows that total protection level for the children was 81.4%. In the ages 1-year to 3-years, an increasing tendency of protection level was evident, ranging from 65.0% to 90.0%. A gradual increasing level of protection was observed in the 15-year-old children. The protection level reached the value of 90.0%. A statistically significant difference was observed for tetanus protection levels, according to the children age; $\chi2 = 112.3$, $p <0.01$ was noted.

All children in this study were protected against tetanus.

In the group of children with insufficient protection, 53.8% were aged 1-year, 23.1% 5-years, 15.4% 3-year, while 7.7% were aged 15-years. In the group of children with sufficient protection, 42.9% were aged 1-year; 21.4% 3-years, and 35.7% 5-years.

Table 3: Distribution of protection levels according to age groups for tetanus

Age (years)	**Protection level (acceptable/full) by age for tetanus (%)**
1	65.0
3	90.0
5	85.0
15	90.0
Total protection level for these age groups	81.4

Source: Authors

In the group of children with acceptable protection, 25% were aged 1-year, 35% 3-years, and 40% 5-years. In the group of children with full protection, 8.7% were aged 1-year, 34.8% 3-years, 17.4% 5-year, and only 39.1% 15-years.

Table 4: Distribution of protective levels according to residence for tetanus

Tetanus IgG (IU/ml)	**Rural Residence children (n=120)**	**Urban Residence children (n=120)**
< 0.10	0	0
0.10 - 0.50	24	18
0.60 - 1.0	18	24
1.10- 5.0	21	39
> 5.0	57	39

Source: Authors

The values in Table 4, indicate that, from the total number of the children included in this study (240), there were few unprotected children against tetanus. Only 42 children (both rural and urban) exhibited insufficient IgG values for protection. However, it is worth mentioning again that, 57 children from rural areas have full levels of protection against tetanus while only 39 urban residence children exhibited full level of protection.

Table 5 shows that the protective level for pertussis reached 69.0%, which indicated efficacy of the vaccine, but not to the degree of diphtheria and tetanus.

A statistically significant difference was observed among different age groups regarding the level of protection for pertussis in this distribution ($\chi 2 = 29.6$, $p < 0.01$).

In the group of children with IgG for pertussis, with insufficient protection levels, 42.9% were aged 5-years and 28.6% were aged 1 and 3 years. In the group of children with good pertussis defensive level, 33.3% were aged 5-years, 26.7% 15-years, and 20.0% were aged 1 and 3 years. In the group of children with IgG optimal protection levels against pertussis, 31.7% were aged 1 and 3 years, 22.0% age 5-years, and 14.6% 15-years.

Table 5: Distribution of protection levels according to age groups for pertussis

Age (years)	Protection level (good - full) for pertussis according to age (%)
1	73.0
3	73.0
5	58.0
15	80.0
Total protection level for these age groups	69.0

Source: Authors

Table 6: Distribution of protective levels according to residence for pertussis

Pertussis IgG (IU/ml)	Rural Residence children (n=120)	Urban Residence children (n=120)
< 14.0	33	9
14.0-18.0	15	42
> 18.0	72	69

Source: Authors

The values in Table 6, indicate that the number of children which showed full protection levels for pertussis in rural (72) and urban residence (69), was almost the same.

As diphtheria and tetanus generally accompany each other in all vaccine combinations in the vaccination scheme, their degree of correlation was assessed, and in these 240 samples the confidence interval (CI) of 95 % was 0.560 to 0.783.

A strong and statistically significant correlation was observed between the level of IgG against diphtheria as well as against tetanus. With the increasing of IgG level towards diphtheria, the level of IgG against tetanus was also increased.

Conclusion

For diphtheria, one could observe a slight predominance slight predominance of safe protection levels of rural areas in terms of long-term in contrast to the urban areas. For tetanus, an increasing trend in protection level was evident after the re-vaccination at the age of 2, with a slight decline observed from then until age 6, when revaccination occurs. The study showed that as children grow older, a gradual decline in protection occurs, until the last re-vaccination at age 14-years.

The vaccination with pertussis vaccine at age of 2 appears to maintain safe protection IgG levels.

Levels of protection for diphtheria and tetanus increased up until 3 years of age (diphtheria: 25.0% at 1 year and then 95.0% at 3 years of age; tetanus: 65.0% at 1 year and 90.0% at 3 years of age). From then on the trend stabilized. The same trend is not observed for pertussis, which presented protective IgG levels of 73.0% from the first year of life. When IgG protective levels were compared between rural and urban samples, differences were only found with diphtheria specific IgG, which was significantly higher in the rural population.

IgG specific levels for diphtheria, tetanus and pertussis referring to our sample are considered acceptable for providing protection in general population. Nevertheless, these levels seem to have been achieved only after booster doses until the third year of life, especially for diphtheria.

Recommendations: Seroprevalence studies such as this study are difficult to conduct in the conditions of Albania; therefore, continuous monitoring becomes a necessity. We recommend special attention be given to vaccination against diphtheria for children living in urban areas and it is also important to supervise the preservation of protection levels for all age groups and geographical areas of Albania.

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