Seroprevalence of *Bordetella pertussis* among a nationally representative sample of Iranian pediatric population: The childhood and adolescence surveillance and prevention of adult noncommunicable disease-V study

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**Background:** Pertussis is a vaccine-preventable respiratory infection and seroepidemiology of the infection could be a marker of the pertussis immunity in a population. In many countries, despite vaccination coverage, high prevalence of pertussis has been observed. The present study aimed to evaluate the immunoglobulin G (IgG) antibody against pertussis and the role of demographic and anthropometric variables on the immunity rate in the Iranian pediatric population to evaluate the impact of existing immunization program in order to envisage future vaccination strategies to prevent infection. **Methods:** In a cross-sectional multi-centric study, 1593 samples of the students aged 7–18 years, who had been enrolled in a national survey (Childhood and Adolescence Surveillance and Prevention of Adult Noncommunicable disease-V), were randomly selected and tested for IgG antibody against *Bordetella pertussis* (BP) by enzyme-linked immunosorbent assay. The age, gender, education, residency, geographical region, and body mass index (BMI) were extracted from the questionnaires of the COSPIAN-Survey. Multiple logistic regression models were used to assess the associations between the variables with the IgG antibody against BP. Data were presented by odds ratio (OR), 95% confidence interval (95% CI) and P values (P): (OR [95% CI]; P). **Results:** Subjects were consisted of 774 boys and 750 girls, with a mean (standard deviation) age of 12.39 (3.03) years. Overall, BP seroprevalence was 59.8%. There were higher BMI values in seronegative ones versus seropositive (18.62 ± 4.07 vs. 18.15 ± 3.94, P = 0.041, 95% CI = 0.23 [0.02–0.92]). However, the categorized BMI for age was not significantly associated with IgG levels (0.27 [0.25–0.29]; 0.27). BP seroprevalence was not significantly different between geographical regions (0.06 [0.05–0.07]; 0.06), genders (1.17 [0.93–1.47]; 0.18), area of residence (1.07 [0.82–1.4]; 0.61), and educational levels (0.94 [0.75–1.19]; 0.62). **Conclusion:** IgG antibody against pertussis was not detected in nearly 40% of the subjects who had history of vaccination against pertussis. It is recommended to monitor the incidence of pertussis in high-risk populations closely and administer a booster dose of acellular pertussis vaccine in adolescents.

**Key words:** Adolescent, child, Iran, pertussis, toxin

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INTRODUCTION

Pertussis or whooping cough is a communicable respiratory illness caused by Bordetella pertussis (BP), which is a Gram-negative microorganism. This infection is endemic in the world; the World Health Organization reported 139,535 cases of pertussis with 89,000 deaths in 2016. Despite affecting all age groups, children are at more considerable risk. Therefore most studies focused on this age group. Although, Vaccination coverage is suitable worldwide children’s mortality rate is still high. According to the findings, lack of vaccination, or delayed vaccination, is one of the most important risk factors.

Pertussis vaccine does not offer lifelong protection, and despite vaccination, the disease is one of the most bacterial illnesses among vaccine-preventable diseases. The first pertussis vaccine, whole-cell pertussis vaccines (wP), had a serious side effect, which caused a decrease in vaccination coverage. The second generation of vaccines, the acellular vaccines (acellular pertussis [aP]), was used in Japan in 1981. The immune response of wP to prevent illness appears to be superior to aP, but aP has better safety. However, wP is still using in many counties including Iran.

Vaccination by triple diphtheria, tetanus and whole-cell pertussis vaccine (DTP) was started in Iran since the 1950s. All Iranian children were vaccinated at the 2nd, 4th, and 6th months of life and then with two booster doses: one at 18 months and another at 6 years.

Despite acceptable coverage of pertussis vaccination in Iran, the illness continues to happen in highly-immunized adolescents. This phenomenon is even seen in industrialized countries, immunoglobulin G (IgG) antibody against pertussis in Iranian children have been evaluated in several studies, and different results were reported.

Without epidemiological studies in the various geographic regions and between different age groups, it is challenging to evaluate immunization programs (such as replacing the wP with aP, adding or elimination of booster doses, and special vaccination groups) and detection of high-risk populations. The epidemiological studies are worthwhile tools to display gaps in population immunity and also highlight potential outbreaks in that area. The study aimed to determine the prevalence of pertussis antibodies in a large pediatric population living in different provinces of Iran and to investigate risk factors of seronegativity among them.

METHODS

Study setting and subjects
To detect the presence of IgG antibody against pertussis in Iranian children and adolescent, in a cross-sectional study which was conducted in 2018, the data and serum samples were obtained from subjects who had participated in the nationwide study entitled Childhood and Adolescence Surveillance and Prevention of Adult Noncommunicable disease (CASPIAN-V). The CASPIAN-V survey had been conducted in 2015 on students in thirty provinces of Iran. Briefly, in CASPIAN-V study was used a probabilistic multistage stratified cluster sampling method to collect samples from the entire Iranian population aged 7–18 years. Selection of schools in provinces had been based on the urban and rural areas, educational levels (primary and secondary), and gender. Clusters had been formed at school levels; each cluster consisted of 10 students and totally, 48 clusters were selected in each province. In each province, 14 out of 48 clusters were randomly chosen for par clinical test.

Overall, 14400 students aged 7–18 years were randomly selected from different schools. Two questionnaires, one on health status and another on health-related behaviors of students and their family, were completed. Both of questioners were valid and reliable. Serum samples were divided in aliquots and stored at −70°C.

Sampling method
In the current study, the sample size was estimated to provide 80% statistical power based on a prevalence rate of 0.47 for pertussis, 2.5% allowed error, 95% z score using the formula: \( n = \frac{z^2 \times p (1-p)}{d^2} \). Finally, 1530 samples were determined. To compensate for probably loss of serums during lab procedure, 1593 samples were included in the study. The samples were selected by random simple sampling method from the serum and information bank and transported to Infectious Diseases and Tropical Medicine Research Center, Isfahan University of medical sciences during 1 h, under refrigerated conditions. Subjects were eliminated from the analysis if they did not have the serum sample volume required for analysis and if information was missing.

Laboratory design
A commercial BP IgG enzyme-linked immunosorbent assay kit (IBL-Hamburg, Hamburg, Germany) with sensitivity 100% was used to determine the prevalence of IgG antibodies against the bacteria in serum samples. The reference values of <16 U/mL, 16–24 U/mL, and >24 U/mL were used, according to the manufacturer’s classification, to determine negative, equivocal, and positive results, respectively. Equivocal samples were double checked, and if their titers were ≤24 U/ml, they were assumed negative.

Anthropometric and demographic assessment
We extracted demographic variables such as age, gender, place of residence (rural/urban), level of
education (elementary/intermediate), and region of the selected samples from CASPIAN-V database. According to a previous national study, Iran has been divided into four regions: North/Northeast, Southeast, West, and Central based on geographic, social, and economic factors. Body mass index (BMI) was used to determine body mass which calculated by weight (kg) divided to the square of height (m). BMI status of students was categorized using z-score chart of the World Health Organization (WHO) for each age and sex. Each subject was considered to be thinness (under-weight), if his/her Z-score was under-2, normal if Z-score was between-2 and 1, overweight if Z-score was between 1 and 2, and obese if Z-score was upper 2.

All of the participants had a history of vaccination against pertussis and had a routine immunization card based on national protocol at 2nd, 4th, and 6th months of life and then with two booster doses: at 18 months and 6 years.

Ethical issues
In CASPIAN-V study, written informed consent that permitted researchers to use samples in the other epidemiological studies had been signed by the students’ parents and a verbal consent had also been taken from the students. The present study protocol was approved by the Ethics Committee of Isfahan University of Medical Sciences, Isfahan, Iran (approval number: IR.MUI.REC.295149).

Statistical analysis
The recorded data were double-entered and analyzed on SPSS software version 15 (SPSS Inc., Chicago, IL, USA). The data were evaluated and managed for the presence of outliers, violations of normality, and missing data. Normality of continues data was evaluated using Shapiro–Wilk test and Q-Q plot.

The categorical data were reported as frequencies and percentages and were analyzed using the Chi-square test. Quantitative normally distributed data were presented as the mean ± standard deviation (SD) and were analyzed using Student’s t-test. The BP seroprevalence categorized by gender, region, BMI, education, and place of residence was tabulated with 95% confidence intervals (CIs). Both of adjusted (adjusted by age and education level) and unadjusted data were analyzed using univariate and multiple logistic regression. \( P \leq 0.05 \) was considered statistically significant for all of reports.

RESULTS

Participants
In the present study, the samples and questionnaires of 1593 children and adolescents with mean (SD) age of 12.39 (3.03) years were evaluated for IgG antibodies against pertussis. Among them, 774 (50.8%) were male and 750 (49.2%) were female. According to the living area, 72.4% of them were living in urban areas. More than 60% of the subjects were at the level of elementary education.

Main outcomes
Totally, 59.8% of the subjects were seropositive for pertussis; no statistically significant difference in mean age was seen between positive and negative samples (positive cases = 12.33 ± 3.09, negative cases = 12.49 ± 2.94, \( P = 0.33, 95\% CI = 0.17 [-0.16–0.49] \)). The highest seropositivity for pertussis was seen at age group 7–9 years and the lowest positive rate was in 10–12 year-old (the differences was not significant). The mean BMI of participants was 18.40 ± 4.17 kg/m²; it was significantly higher in negative samples than in positive ones (18.62 ± 4.07 vs. 18.15 ± 3.94 years, respectively, \( P = 0.041, 95\% CI = 0.23 [0.02–0.92] \)). The lowest (54.5%) and highest (63.1%) seropositivity were observed in the central and west regions of Iran, respectively; however, the differences in region areas were not significant. No significant differences were seen between IgG Ab and gender, education, or area of residence [Table 1].

In multivariate logistic regression analysis, the age and education were considered as cofounder variable and were adjusted in the analysis. As shown in Table 2, when age and education level were adjusted in multivariate analysis, no significant association was observed between IgG Ab against pertussis with gender, BMI, the living area, and region.

DISCUSSION
This study is one of the most extensive seroprevalence studies on pertussis conducted in the Middle East and North Africa. According to our results, the pertussis antibody was detected in 59.8% of students aged 7–18 years in Iran. A previous study reported a seroprevalence of 47% in 6–17 year students in Ahvaz, a province in the south of Iran. In a study in Tehran, pertussis seroprevalence was reported to be 39.1%, 46.3%, and 64.5% among subjects at age groups 6–10, 11–15, and 16–20 years, respectively. In a cross-sectional survey in Iran, antibodies against pertussis toxin (PT) were detected in 44.2% of subjects aged 10–25 years. The lowest and highest antibodies were at age groups 10–14 and 21–25 years, respectively.

Our results showed that almost 60% of our subjects had previous contact with BP, either via vaccination or contact with the infected person. Antibody starts to decline 9 months after exposure with antigen and period of immunity against pertussis is 4–12 and 4–20 years after vaccination and natural
infection, respectively. There is not a life-long immunity for the vaccine.[2] A comparison of our results with other studies previously performed in Iran showed that less population is at risk of infection. It seems that we experience better condition than before. Data of similar studies in other countries reveal different results. In a study in Taiwan, the positivity rate of anti-pertussis IgG reported 42.5% among 2782 school children from 58 schools.[18] In Japan, pertussis IgG antibodies were detected in 47.1% and 60% of children at age group 6–7 and 12–13 years, respectively, in 2014 and 73% at age group 18–19 years in 2013. In 2015, those were 37.4%, 61.3%, and 75.7% for age group 6–7, 12–13, and 18–19 years, respectively.[19] A survey in Estonia showed that 48.7% of children younger than 18 years had anti-PT IgG in undetectable concentrations. In this study, only 3.6% of subjects aged 9–14 years were positive for anti-PT IgG.[20] The type of vaccine, the interval between the last dose of the vaccine and sampling time, number of booster doses, stage of pertussis epidemic cycle is different from a study to another. So, it is difficult to compare results between various countries. Hence, it is difficult to compare results between various countries.

It should be noted that there is no agreement on the accepted level of protection. Furthermore, some studies reported that immunity against BP infection is multifactorial. The presence of antibodies alone is not enough to protect, and CD4+, B-cells, and T-cells also have a role in protection.[20] A practical method to end the circulation of bacteria is still unknown. A survey demonstrated that vaccine protects against the disease, but it is not fruitful preventing transmission.[21] Decreased immune response to pertussis after a routine vaccination makes children and adolescents a source of transmitting the infection to young infants, who are under 6 months age.[22]

| Table 1: The association of demographic data with seropositivity for anti-Bordetella pertussis antibodies in Iranian pediatrics |
|-------------------------------------------------------------|--------|-----------------|-----------------|----------|
| Variables                                                  | Samples tested (%) | Seropositive samples (%) | Odds ratio (95% CI) | P*      |
| Age (years)                                                |        |                  |                  |         |
| 7-9                                                        | 282 (19.8) | 188 (66.7)       | 0.3 (0.27-0.32)   | 0.29    |
| 10-12                                                      | 469 (32.9) | 285 (60.8)       |                  |         |
| 13-15                                                      | 386 (27)  | 235 (60.9)       |                  |         |
| 16-18                                                      | 290 (20.3) | 188 (64.8)       |                  |         |
| Missing data                                               |        | 166              |                  |         |
| Sex                                                        |        |                  |                  |         |
| Female                                                     | 750 (49.2) | 441 (58.8)       | 0.87 (0.71-1.07)  | 0.2     |
| Male                                                       | 774 (50.8) | 480 (62)         |                  |         |
| Missing data                                               |        | 69               |                  |         |
| Area of residence                                          |        |                  |                  |         |
| Urban                                                      | 1054 (72.4) | 649 (61.6)     | 0.90 (0.71-1.13)  | 0.36    |
| Rural                                                      | 402 (27.6) | 237 (59)        |                  |         |
| Missing data                                               |        | 137              |                  |         |
| Education level                                            |        |                  |                  |         |
| Elementary                                                 | 790 (60.9) | 496 (62.8)       | 0.94 (0.75-1.19)  | 0.62    |
| Intermediate                                               | 508 (39.1) | 312 (61.4)       |                  |         |
| Missing data                                               |        | 295              |                  |         |
| BMI for age                                                |        |                  |                  |         |
| Under-weight                                               | 141 (10.9) | 97 (68.8)        | 0.27 (0.25-0.29)  | 0.27    |
| Normal                                                     | 921 (71.5) | 572 (62.1)       |                  |         |
| Over-weight                                                | 158 (12.3) | 93 (58.9)        |                  |         |
| Obese                                                      | 69 (5.4)  | 40 (58)          |                  |         |
| Missing data                                               |        | 304              |                  |         |
| Region                                                     |        |                  |                  |         |
| North/Northeast                                            | 309 (19.9) | 185 (59.9)       | 0.06 (0.05-0.07)  | 0.06    |
| Central                                                    | 396 (25.4) | 216 (54.5)       |                  |         |
| West                                                       | 612 (39.3) | 386 (63.1)       |                  |         |
| Southeast                                                  | 239 (15.4) | 143 (59.8)       |                  |         |
| Missing data                                               |        | 37               |                  |         |

*Resulted before adjusting in univariate logistic regression analysis. CI=Confidence interval.
A considerable number of Iranian children and adolescents with a positive history of vaccination against pertussis did not have IgG antibodies to pertussis. As cellular immunity might have a role in preventing the infection, it is not possible to determine high-risk population, and further studies on the incidence of the disease are warranted. It seems that administering a booster dose of aP vaccine in adolescents is necessary to protect them from natural infection.

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Conflicts of interest
There are no conflicts of interest.

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Table 2: The adjusted association between the demographic data of Iranian pediatrics with seroprevalence of Bordetella pertussis

| Variables          | Odds ratio (95% CI) | P*          |
|--------------------|--------------------|-------------|
| Sex                |                    |             |
| Boy                | 1.17 (0.93-1.47)   | 0.18        |
| Girl               | 1.00 (reference)   |             |
| Area of residence  |                    |             |
| Urban              | 1.07 (0.82-1.4)    | 0.61        |
| Rural              | 1.00 (reference)   |             |
| BMI for age        |                    |             |
| Under-weight       | 1.00 (reference)   |             |
| Normal             | 1.00 (0.85-2.88)   | 0.15        |
| Overweight         | 1.18 (0.72-1.95)   | 0.52        |
| Obese              | 1.05 (0.59-1.88)   | 0.86        |
| Region             |                    |             |
| North-Northeast    | 0.89 (0.61-1.29)   | 0.53        |
| Central            | 0.76 (0.51-1.15)   | 0.19        |
| West               | 0.75 (0.49-1.15)   | 0.19        |
| Southeast          | 0.89 (0.61-1.29)   | 0.53        |

*Resulted after adjusting by multiple logistic regression analysis. CI=Confidence interval

group 16–18. Since all Iranian children were vaccinated with a booster dose DTP at the 6-year-old, it is expected that the highest rate was observed in the age group 7–9 years. We saw an increase in the age group 16–18 too; it may be due to the acquisition of natural infection BP.

Other similar studies in the world have shown different results. In a study in Mexico that was performed on subjects aged 1–95 years, pertussis seroprevalence showed a significant difference with sex and age, but regions and socioeconomic status were not significant factors. IgG antibodies against BP were assessed in a study in Singapore. No significant differences were seen between the two genders. In a cross-sectional study in the north of Iran, IgG antibodies levels were checked, and results showed the lowest level among school-aged. The controversy in the results of studies may be due to different methodology of studies, samples, and the context of societies. In the present study, samples were collected from different provinces of Iran; therefore, it seems our results can be expanded for other regions with similar conditions.

CONCLUSION

A considerable number of Iranian children and adolescents with a positive history of vaccination against pertussis did not have IgG antibodies to pertussis. As cellular immunity might have a role in preventing the infection, it is not possible to determine high-risk population, and further studies on the incidence of the disease are warranted. It seems that administering a booster dose of aP vaccine in adolescents is necessary to protect them from natural infection.
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