Journal of Advanced Research (2016) 7, 105–111

Cairo University

Journal of Advanced Research

ORIGINAL ARTICLE

# Assessment of antibody level and avidity against *Bordetella pertussis* in a cohort of Egyptian individuals aged 1–18 years

**Nihal M. Ibrahim [a,*], Ebtsam M. El-kady [b], Somiaa A. Eissa [c], Ahmed F. Wahby [a]**

[a] *Department of Molecular Biology, National Research Center, Dokki, Cairo, Egypt*
[b] *Department of Microbial Biotechnology, National Research Center, Dokki, Cairo, Egypt*
[c] *Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Egypt*

ARTICLE INFO

*Article history:*
Received 8 July 2014
Received in revised form 28 January 2015
Accepted 14 March 2015
Available online 21 March 2015

*Keywords:*
Whooping cough
*B. pertussis*
Antibody avidity
Vaccination

ABSTRACT

Pertussis specific antibodies were studied with respect to quality and quantity in a cohort of apparently healthy Egyptian children and adolescents, with their age range between 1 and 18 years, in an attempt to get a close and clear insight into the current humoral immunization status in this specified group and to try find a relation between the antibody levels and their avidities in eradication of this devastating infectious disease. Our results showed that avidity increase was most marked in young school children (6–8 years) where it seemed to reach a plateau in older children and adolescents. Antibody titer was highest in toddlers (1–2 years) and young school children (6–8 years) groups, most probably following vaccination and/or booster doses. Among children aged 1–5 years, 28% had highly avid and 50% had high titer antibodies, whereas in adolescents aged 13–18 years, 70% had highly avid antibodies and only 30% had high titer antibodies. The results clearly demonstrated that while levels of anti-*Bordetella pertussis* (*B. pertussis*) antibodies wane with growing age, the avidity seems to increase, to a plateau, irrespective of further antigen exposure in a pattern showing complete independence of avidity on concentration. The present study draws attention to the importance of avidity measurements, together with conventional ELISAs, for evaluating immunity against pertussis. Being based on a limited sample size, it could open doors for larger-scale surveys to be possible indicators for the need and timing of booster vaccination doses among Egyptians.


* Corresponding author. Tel.: +20 222668203, mobile: +20 1226413601, +20 1282808675.
E-mail address: n-hal@hotmail.com (N.M. Ibrahim).
Peer review under responsibility of Cairo University.

Production and hosting by Elsevier

## Introduction

Pertussis (whooping cough) is an acute disease of the upper respiratory tract caused by the gram negative bacillus *Bordetella pertussis* (*B. pertussis*). Bacterial pneumonia or respiratory distress is the usual cause of death [1]. Worldwide, pertussis remains one of the top ten causes of

http://dx.doi.org/10.1016/j.jare.2015.03.002
2090-1232 

vaccine-preventable deaths in children under 1 year with an estimated 30–50 million cases and as many as 300,000 pertussis related deaths annually, 90% in developing countries and mostly in infants [2]. In 2000, disability-adjusted life years from pertussis (12.7 million) exceeded those of lung cancer (11.4 million) and meningitis (5.8 million) [3].

Despite high childhood vaccination coverage, since the universal implantation of the whole cell vaccine in the 1940s, pertussis has reemerged as a public health problem worldwide in the past 2–3 decades [4–7]. Waning immunity following infant vaccination and reduced opportunity of pertussis for boosting immunity due to reduced circulation of *B. pertussis* contribute to increased susceptibility to pertussis infection and disease in adolescents who are the main source of infection to vulnerable infants too young to be vaccinated [8–10]. Estimates of the duration of protection following whole cell pertussis vaccination range from 4 to 12 years and following acellular vaccination is approximately 5–6 years [11].

Antibody decay rates and mathematical modeling [12–14] suggest that repeated doses of pertussis vaccines will be needed to maintain protection against pertussis. Moreover, many developed as well as developing countries including the Eastern Mediterranean region [15] have recommended adult vaccination against pertussis. In Egypt, the whole cell vaccine combined with diphtheria and tetanus toxoids (DPT) has been introduced in the schedule of compulsory vaccination for Egyptian children at 2, 4, and 6 months followed by a booster dose in the second year of life [16]. Comparison of pertussis incidence between countries is problematic due to differences in case definition, access to diagnostic tests, clinician awareness and differences in immunization strategies [10]. In many developing countries, identification of pertussis is still limited by patient and physician awareness and the limited sensitivity of diagnostic tests although the WHO estimates demonstrated that these countries have the highest disease burden [17].

The humoral immune response to a specific antigen comprises the magnitude of antibody as well as the affinity of an antibody to its antigen [18]. Clinically, serum antibody level is a useful parameter that could detect the presence of infection and the magnitude of protective antibodies against a certain pathogen following natural infection or vaccination. Qualitative parameters, affinity and avidity, mainly measure the binding strength of a pathogen to specific antibodies thereby determine the efficiency of the circulating antibodies and their ability to induce protection against a disease [19].

Antibody avidity characterizes the functional affinity of multivalent antibody binding with multivalent antigens [18]. Antibody avidity represents the functional measure of affinity maturation of antibodies and is correlated with long term immunity [20,21] and possibly protection against pertussis disease [22]. Antibody levels increase shortly after antigen contact while increase in avidity is much slower [23,24] and appears to be an age dependent process observed from six months onwards [25] and is present at significant levels at 4 years old in children [26,27].

As the antibody quantity and quality are good serological markers of vaccine efficacy, the present study was designed to assess the antibody titer levels and avidities in a cohort of Egyptian children and adolescents (1–18 years) which could be an attempt to find a relation between these two criteria and show how much both parameters would contribute to define the immunization status, with respect to age, against pertussis in the individuals under test.

## Subjects and methods

### *Serum samples*

Serum samples were randomly collected from children and adolescents aged 1–18 years who admitted KIDS hospital, Al-Mohandeseen, Cairo, Egypt, in the period between June 2012 and December 2012 ($n = 59$) and in January 2015 ($n = 33$). Two samples collected from infants 5 and 6 days old were used as control. Most individuals participating in the study suffered minor to moderate health problems although no one had been diagnosed with underlying pertussis disease or other respiratory infections. All participants had been immunized according to the Egyptian national immunization program at 2, 4, 6 months followed by a booster at 18 months. The inclusion criteria include apparently healthy immunized members and those who had suffered transient weakness or instability but with no underlying acute or chronic disease, while the exclusion criteria include individuals who suffer chronic diseases, acute illness or those who require long therapy especially individuals treated with steroids, chemotherapeutics, immunoglobulins or other immunosuppressive drugs, in addition to those who recorded antibody titers $< 50$ or avidity values $< 0.5$.

*The study was completely a random clinical trial and was approved by the research ethics board at KIDS hospital, Al-Mohandeseen, Cairo, Egypt. An oral informed consent was obtained from the parents before starting the protocol.*

### *Antibody titer determination by ELISA*

Serum anti-pertussis antibody titer was measured with the standardized ELISA [28] with minor modifications. Briefly, 96 wells-microtiter plates (Dynatech) were coated overnight at 4 °C with 100 µl of pertussis antigens (dil 1:1000) in the coating buffer (0.05 M carbonate buffer pH 9.6). The pertussis antigens contained equal volumes of the whole cell *B. pertussis* strains 134, 509, and 165 that were kindly provided by the VACSERA authorities. The plates were washed three times with PBS-T buffer (100 mM PBS pH 7.5 containing 0.05% Tween 20) and incubated overnight with 150 µL/well of the blocking buffer (100 mM PBS pH 7.5 containing 0.5% gelatin). Serial dilutions of the tested human sera (100 µL) in the PBS-T were dispensed into duplicate wells and incubated for 2 h at room temperature then overnight at 4 °C. After wash, anti-human alkaline phosphatase conjugate diluted in PBS (1/2500) was added (100 µl/well) and incubated for 2 h at 37 °C. The plates were washed thoroughly for 3–5 times with PBS-T buffer before allowing them to react with 100 µl/well of the substrate solution (4 mµ P-nitro phenyl phosphate, P-NPP, 1 mµ $MgCl_2$ in 1 µ diethanolamine, pH 10). The reaction was allowed to proceed for 30 min at room temperature in the dark before the addition of 1 N NaOH (50 µL). The developed ODs were measured at 490 nm in a Micro ELISA Reader Photometer. A reference serum was used to correct from plate-to-plate errors and the antibody titer was calculated as the antibody dilution that gives an OD of 0.5 absorbency.