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

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**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.

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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 μM P-nitrophenyl phosphate, P-NPP, 1 μM MgCl2 in 1 μl 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.
Antibody avidity tests

Antibody avidity was determined according to [29] with little modifications. Briefly, ELISA plates were coated with pertussis antigens and blocked with gelatin as described under the previous section. The different human sera (dilution 1:30) were allowed to bind the pertussis toxin (1:1000) in the ELISA plates. The formed antigen–antibody complexes in twelve wells were used to determine the avidity index of one serum. Following washing of the plates (3 times) with PBS-T buffer, 100 μL of NH₄SCN in PBS pH 6 were added at five different concentrations (0.25 M, 0.5 M, 1 M, 2 M and 3 M) leaving two wells filled with the dilution buffer alone, without NH₄SCN, as a control. After 15 min, the plates were washed with PBS-T and processed as previously described under the standard ELISA in the previous section. A plot of the percentage binding (OD in presence of NH₄SCN/OD in absence of NH₄SCN × 100) versus the NH₄SCN concentration was established and used for interpretation of the avidity index. The NH₄SCN concentration that develops 50% of the binding in the NH₄SCN-free sample was considered as the relative avidity index for the tested serum.

Results

The humoral response against B. pertussis in Egyptian children and adolescents 1–18 years

Fig. 1 shows the avidity and antibody titer concentrations of 92 sera samples obtained from randomly selected cohort of Egyptian children and adolescents 1–18 years old. The high variation coefficient (CV) values reflect the great variations in antibody response with respect to magnitude and/or avidity among individuals of the same age (Table 1).

Relationships between antibody avidity, titer and age

Both antibody titers and avidity associated weakly with age. While antibody avidities to whole cell pertussis antigens correlated positively ($R = 0.368$, Fig. 2A), antibody levels were inversely associated ($R = -0.621$, Fig. 2B) with age. Very poor, almost no, correlation could be detected between avidity and titer of pertussis antibodies ($R = -0.068$, Fig 2C).

Behavior of avidity and titer of anti-pertussis antibodies over the different age groups

The behavior of avidity and titer over five different age groups were examined as shown in Fig. 3. It is evident that the avidity tends to smoothly increase by growing age until a plateau was reached in school children and adolescents. On the contrary, ELISA titer showed two maxima: the first in toddlers (1–2 years), and the second in young school children (6–8 years). Assuming that high avid antibodies are those registering avidity indices ≥1.2 and high level antibodies are those with ELISA titers ≥200, the results showed that 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.
Table 1  Mean avidity indices and titers of antibodies specific to pertussis in the different age participants.

| Group              | Age   | Number (participants) | Avidity index (Mean ± SE) | CV% | ELISA titer (Mean ± SE) | CV% |
|--------------------|-------|------------------------|---------------------------|-----|-------------------------|-----|
| Toddlers           | 1     | 8                      | 0.951 ± 0.059             | 6.27| 273.99 ± 62.012         | 22.6|
|                    | 2     | 7                      | 0.976 ± 0.047             | 4.81| 334.47 ± 47.096         | 14.1|
| Pre-school children| 3     | 7                      | 1.328 ± 0.239             | 17.99 | 192.46 ± 40.419       | 21  |
|                    | 4     | 4                      | 1.165 ± 0.168             | 14.42| 190.67 ± 27.942        | 14.6|
|                    | 5     | 6                      | 1.038 ± 0.143             | 13.78| 143.53 ± 24.824        | 17.3|
| Young school children| 6   | 7                      | 1.195 ± 0.179             | 14.97| 263.28 ± 51.614        | 19.6|
|                    | 7     | 5                      | 1.255 ± 0.183             | 14.59| 480.26 ± 40.147        | 8.4 |
|                    | 8     | 5                      | 1.841 ± 0.381             | 20.7 | 273.12 ± 51.875        | 19  |
| Old school children | 9    | 9                      | 1.437 ± 0.187             | 13.01| 189.32 ± 24.774        | 13.1|
|                    | 10    | 6                      | 1.208 ± 0.176             | 14.56| 158.35 ± 24.198        | 15.2|
|                    | 11    | 6                      | 1.54 ± 0.269              | 17.46| 176.97 ± 34.521        | 19.5|
|                    | 12    | 2                      | 1.55 ± 0.05               | 3.22 | 51.76 ± 3.513          | 6.8 |
| Adolescents        | 13    | 4                      | 1.186 ± 0.278             | 23.4 | 108.46 ± 17.604        | 16.2|
|                    | 14    | 2                      | 1.32 ± 0.63               | 47.7 | 128.07 ± 10.075        | 7.8 |
|                    | 15    | 3                      | 1.078 ± 0.521             | 48.3 | 180.14 ± 52.62         | 29.2|
|                    | 16    | 5                      | 1.439 ± 0.069             | 4.8  | 198.38 ± 34.363        | 17.3|
|                    | 17    | 2                      | 2.065 ± 0.135             | 6.5  | 197.42 ± 37.32         | 18.9|
|                    | 18    | 4                      | 1.666 ± 0.277             | 16.6 | 175.02 ± 54.784        | 31.3|

\[a \text{ SE, the standard error among the stated number of participants.}\]

\[b \text{ CV\%, the coefficient of variation} = \text{SE/mean} \times 100.\]

Fig. 2  Correlation between avidity indexes and serum titer levels of antibodies to whole cell pertussis antigens in relation to age (A and B, respectively) and in relation to each other (C). Linear regression lines are shown as the associated $R$ values.
The present study was designed to elucidate the humoral immunity, with respect to antibody quality and quantity, in a randomly chosen cohort of Egyptian children and adolescents in an attempt to find a relation between these two criteria and how much both would contribute to define the immunization status, with respect to age, against pertussis in the individuals under test.

According to our results, both antibody levels and avidities associated (albeit weakly) with age; while avidity correlated positively, antibody levels wane with growing age. The lowest antibody levels were recorded in adolescents (13–18 years) in whom the highest avid antibodies were most prominent. The percentage of individuals having high avid antibodies increased from 28% in children (1–5 years) to about 70% in adolescents (13–18 years), whereas the proportion of individuals with high titer antibodies decreased from 50% to 30% along the same previous age groups.

Nowadays, the immunization schedules vary from country to country [10]. The Egyptian national program for obligatory vaccination includes immunization at 2, 4, 6 and 18 months [30]. As it is well known that antibody levels substantially increase following vaccination and fast decrease after then [31–33], accordingly it was completely accepted that the highest antibody titers were registered in toddlers (1–2 years), but it was surprising to detect such high levels of anti-pertussis antibodies, comparable to those recorded in toddlers, in young school children (6–8 years) as well. This observation could reflect the culture and traditions of Egyptian parents and their inherent fears of infectious diseases that enhance them to optionally revaccinate their children at school entry even against diseases not included in the national immunization program. Otherwise, the possibility that the measured anti-pertussis antibodies were due to silent infection cannot be excluded, especially when we know that antibody concentrations are higher after infection than following vaccination [22].

Although almost no correlation was observed between antibody level and avidity, very low avid antibodies (<0.5 M NH₄ SCN) were mostly of low titer. This might be attributed to that lower proportions of low avid antibodies are bound to the coating antigen which means that the actual quantification of low avid antibodies could be missed. Likewise, very low titer antibodies could readily be eluted by low NH₄ SCN concentrations that made the detection and analysis of high avid antibodies preferentially favoured [34]. The degradation of antibodies during storage cannot, however, be excluded.

The values of avidity indices could hardly exceed 2 M NH₄ SCN which is too low compared to other antigens tested in our laboratory under the same conditions. This was most likely attributed to the reduction of antibody binding due to susceptibility of the whole cell pertussis antigens to high NH₄ SCN concentrations with subsequent denaturation and elution. Moreover, the coating antigen could be different from that found in physiological conditions [35], even though we used three strains of B. pertussis as coating antigens. Furthermore, ELISA tests that measure antibody level to the whole cell antigens are generally of low sensitivity and specificity compared to that measured against individual pertussis antigens [36]. Nevertheless, avidity index is not an absolute value but rather a relative measure and strongly varies as a function of the assay conditions [18].

The present study was completely a randomized and unsystematic study, so that the results have to be interpreted with

![Graph showing behavior of avidity indices and ELISA titer over five age groups.](image)

**Fig. 3** Behavior of avidity indices and ELISA titers over five age groups. Each point and column represents the mean value of avidities and titer levels, respectively, for the participants in each age group under test.

(Table 2). On the other hand, it seemed that school children (6–12 years) had an acceptable amount of antibodies, with respect to quantity and quality, as 55% had high avid antibodies and 52.5% had high titer antibodies. However, it is not necessary that the same individuals with high titer antibodies are those carrying high avid antibodies.

**Discussion**

**Table 2** Distribution of high avid and high titer antibodies specific to whole cell pertussis antigens over the tested groups.

| Years  | Age group          | Number | High avid antibodies | %   | High titer antibodies | Number | %   |
|--------|--------------------|--------|----------------------|-----|-----------------------|--------|-----|
|        |                    |        | *High avid antibodies* |     | *High titer antibodies* |        |     |
| 1–5    | Toddlers (1–2 Yrs) | 32     | 9                    | 28.125 | 16                    | 50     |     |
| 6–12   | Young school age children (6–8 Yrs) | 40     | 22                   | 55  | 21                    | 52.5   |     |
| 13–18  | Adolescents (13–18 Yrs) | 20     | 14                   | 70  | 6                     | 30     |     |

* Antibodies with avidity indices ≥ 1.2.  
* Antibodies with ELISA titers ≥ 200.
Inversely, higher titer antibodies do not always imply (entail) higher quality of antibodies. So that we can come to a conclusion that the decrease in antibody levels in adolescence is compensated by selection of high avid antibodies that might confer some sort of naturally acquired protection against the disease in this age group. Accordingly, we can cautiously say that Egyptians have acquired high immunity in childhood due to active immunization and in teenager hood due to avidity maturation. This, however, does not rule out the presence of participants of low titer and avidity. Because serologic levels of protection have not yet been established [7,42], we cannot decide whether these participants are compromised by the reduction in antibody titer and/or avidity. Further studies have to be conducted on well-known history persons to show how well these immunity parameters correlate with clinical protection.

**Conclusions**

The present report opens doors for further studies on immunity against pertussis, and may be other pathogens, in Egypt and highlights the importance of avidity measurements, together with conventional ELISAs, for evaluating immunity against diseases to enrich the information given about antibodies. However, a wider survey had to be done including larger number of participants from different social levels and different environments before we are able to recommend any boosters in the national immunization program.

**Conflict of interest**

The authors declare that there is no conflict of interest.

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Humoral response against pertussis among a cohort of Egyptians

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