

## EVALUATION OF SERUM OPSONIC CAPACITY AGAINST *BORDETELLA PERTUSSIS* BY CHEMILUMINESCENCE

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### ABSTRACT

Serum opsonic capacity against *B. pertussis* was studied by using quantitative chemiluminescence (CL), a method known to have several advantages over conventional methods in evaluating opsonization and phagocytosis. Sera from unvaccinated infants was shown not to contain opsonins against *B. pertussis* and in unvaccinated infants suffering from whooping cough, no opsonins were detected. In adults fully vaccinated during childhood, antibody titers decreased with time. Therefore, antibody transfer to infants is negligible. The CL assay is simple, rapid, and reproducible, offering new possibilities to evaluate humoral immune mechanisms and phagocytosis in whooping cough.

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### INTRODUCTION

Despite widespread use of an effective whole cell vaccine for more than 40 years, outbreaks of pertussis continue to be reported.<sup>1</sup>

Pertussis (whooping cough) is an endemic and epidemic disease caused by *B. pertussis*. The disease has been and still is a major cause of morbidity and mortality in young children throughout the world. The WHO estimates that 600,000 deaths due to pertussis occur yearly, and virtually all of these deaths occur in unimmunized infants.<sup>1,2</sup> Pertussis immunization induces serologic responses which can be measured by several different assays. Pertussis serology is of value in the diagnosis of whooping cough and may be helpful in evaluating the efficacy of vaccination.<sup>4</sup>

There is accumulating evidence that subclinical infection with *Bordetella pertussis* occurs in immunized individuals who are exposed to persons with pertussis during epidemics and through household contact.<sup>1,7-11</sup> The purpose of this study was to investigate the efficacy of whole cell vaccination in a highly-immunized Iranian population.

### MATERIALS AND METHODS

In this study we selected 260 subjects. 50 adults vaccinated with DTP vaccine in childhood served as a control group. 70 unvaccinated neonates 5-30 days old, and 140 fully vaccinated children 2-4 years of age were entered in the study. Subjects with a history of 3 or more pertussis immunizations were considered fully immunized.

#### Reagents

Hanks' balanced salt solution (HBSS buffer pH 7.4) without phenol red was purchased from Flow Laboratories. Luminal (10 mM stock solution in 0.2 M sodium borate buffer pH 9.0) was from Sigma.

#### *Bordetella pertussis* bacteria

Whole cell suspensions of killed bacteria (a mixture of strains 1.2.3 & 1.2) obtained from the vaccine production unit of the National Public Health Institute (Helsinki, Finland) and Razi Institute in Iran were washed twice in HBSS supplemented with 0.1% gelatin.<sup>4</sup>

**Table I. Comparison of neonates and control groups.**

Subjects	Age	Mean+SD	T	P
Neonates (70)	5-30 days	9.49+2.76	63.83	< 0.001
Control (50)	18-25 yrs	59.99+19.5	73	< 0.001

The comparison of T & P between neonates and vaccinated children (P < 0.001) showed a significant difference between the two groups.

**Phagocytic cells**

Unfractionated leukocytes were obtained from the buffy coat layer of sedimentary erythrocytes prepared with 1.5% dextran at 37°C for 90 min. Leukocytes were separated from plasma by centrifugation and suspended in HBSS buffer after washings.

**Opsonization**

For opsonization, 50 µl of serum in different dilutions and 50 µl of the *B. pertussis* cell suspension (1×10<sup>9</sup>) were incubated in 4 ml polypropylene tubes at 37°C for 30 min and then cooled below room temperature. The serum specimens were stored at -20°C until analysis was performed.

**Chemiluminescence assay**

An automated luminometer set up (LK Wallac 1251 luminometer connected to an Olivetti M20 microcomputer) was used for continuous and simultaneous measurement of up to 25 samples. Cold suspensions of separated leukocytes (4×10<sup>6</sup> cells in 400 µl of HBSS buffer, including 4×10<sup>-4</sup> M luminol and 0.1% gelatin) were added to the opsonization vials. The CL emission commenced when the contents of the vials reached the necessary temperature.

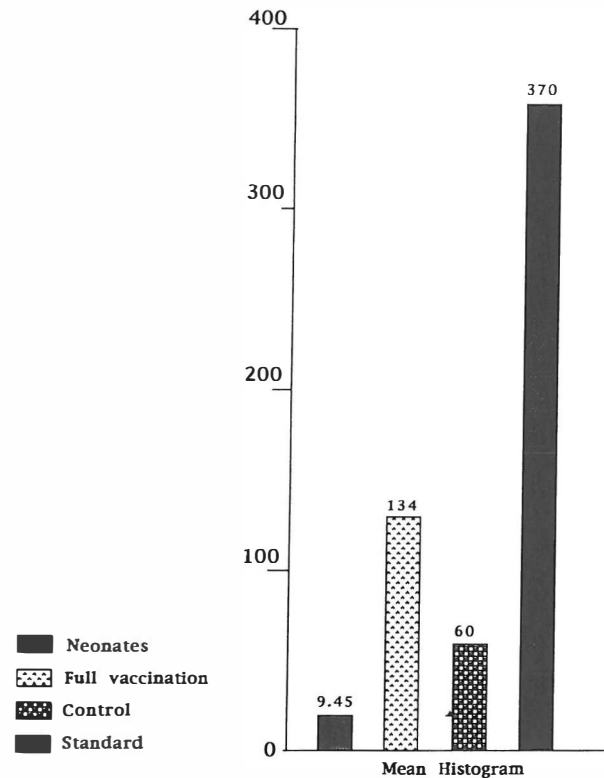
**RESULTS**

The data indicate the CL emission from phagocytosis of *B. pertussis* cells opsonized in diluted sera of whooping cough patients was far more intensive than that of unvaccinated controls. Moreover, CL

**Table II. Comparison of standard and fully vaccinated groups.**

Subjects	Age	Mean+SD	T	P
Fully vaccinated (140)	2-4 yrs	133.99+140.0	43.06	< 0.0001
Standard Pertussis Immunoglobulin 160mg/ml	2-4 yrs	370+90	42.9	< 0.0001

The comparison of T and P in standard and controls (p<0.0001) showed a significant difference between the two groups.



**Fig. 1. Comparison of antibody in neonates, fully vaccinated, control, and standard groups by chemiluminescence**

emission was not observed when unopsonized cell (1×10<sup>9</sup> cells incubated in HBSS buffer instead of serum) were incubated with leukocytes. These factors led us to use the peak CL value (reached in about 30 min) as a relative measure of opsonins in a given serum dilution after deducing the corresponding background CL obtained from serum alone.

In the neonate group the mean+SD of antibody titer by CL was 9.49 + 2.76 mV. The antibody titer by CL in fully vaccinated children was mean + SD = 133.99 + 140.0 mV.

The comparison of T and P between neonates and vaccinated children (T = 73, P < 0.001) showed a significant difference between the two groups.

In the control group the antibody titer was mean +

SD = 59.99 + 19.5 mV. This shows that antibody titers in adults decrease by time, leaving a very low titer to transfer to infants.

We used pertussis immunoglobulin (160 mg/ml) as a standard. The estimation of T and P in standard and controls (T = 42.9, P < 0.0001) showed a significant difference between the two groups.

In this study we also screened 6 children with typical symptoms of pertussis and nasopharyngeal cultures were positive for *B. pertussis* in all six.

### DISCUSSION

Our data showed that CL assays can be performed for the estimation of pertussis opsonins. Unvaccinated infants, healthy vaccinated adults and whooping cough patients have different capacities to opsonize *B. pertussis*.

Neonates have no antibodies against *B. pertussis*. In fully vaccinated children, protecting titers last for about four years, and in adults who were vaccinated in childhood, antibody titers are low. Therefore, a protective vaccine with high potency and efficacy, low toxigenicity and side effects<sup>3,6</sup> must be prepared, and if this prepared vaccine could be injected in adults and pregnant women, antibodies against *B. pertussis* could possibly cross the placenta, thus protecting infants before vaccination and decreasing neonatal mortality and morbidity. In December 1991, the FDA licensed Acel-immune, an acellular pertussis component vaccine. This preparation contains 40 µg of protein; approximately 86% of this protein is FHA, 8% is PT, 4% is M 69000 (an outer membrane protein) and 2% is type 2 fimbriae.<sup>2</sup>

To summarize, whooping cough induced marked and specific serum opsonic activity against *B. pertussis* which was easily detected using the automatic CL

assay. This method proved to be a useful tool for studying the activity of the immune system in this disease.<sup>4</sup>

Our assay system may also be useful in the evaluation of new acellular vaccines.

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