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# Frequency of IgG Antibodies to Pertussis Toxin Antigen among Antenatal Women Attending a Tertiary Care Hospital in Hyderabad

Dr. Srinivas N[1], Dr. Y. Kathyayani[2*]

[1]Assistant Professor of Microbiology, Osmania Medical College, Hyderabad India
[2]Assistant Professor of Microbiology, SV Medical College, Tirupati Andhra Pradesh India

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***Corresponding author:** Dr. Y. Kathyayani

**Abstract** **Original Research Article**

***Introduction:*** Pertussis is a vaccine preventable disease. Recently the is a rise in number of cases of Pertussis in developed countries. Under Universal Immunisation Programme in India two doses of TT vaccine are administered. One dose at 5 months of gestation or as soon as detection of pregnancy and the other 4 weeks apart. American College of Obstetricians and Gynecologists recommends a dose of Tdap during pregnancy. CDC also recommends a dose of Tdap vaccine to all pregnant women between 27- 36 weeks of gestation. This study has been conducted to know the level of IgG antibodies against Pertussis toxin in antenatal women since there is a dearth of literature in this aspect. ***Materials & Methods:*** Blood samples have been collected from 91 Antenatal women attending outpatient department of Tertiary Care Hospital, Hyderabad after obtaining consent. Serum has been separated and stored at -20$^0$C. ELISA has been put up against Pertussis toxin and Serum Anti-Pertussis toxin IgG antibody levels were analysed. ***Results:*** A value of >30 IU/ml was accepted as potential protective concentration, 20 -30 IU/ml as borderline &<20 IU/ml as negative. We found that the percentage of protected, borderline and unprotected antenatal women respectively were 16.48%, 9.89% & 73.62% for anti PT. ***Discussion:*** Most pregnant women in this study demonstrated low anti PT IgG. This finding suggests that a significant number of women & newborn were susceptible to Pertussis. ***Conclusion:*** Our results explained the susceptibility for Pertussis among pregnant women and also newborn born to them & supported the requirement for a Pertussis booster vaccine during pregnancy along with Tetanus and Diphtheria which may provide passive seroprotection in newborns during first months of life decreasing severity of illness and providing seroprotection.

**Keywords**: Pertussis, Tdap vaccine, ACOG, CDC.



## INTRODUCTION

Pertussis (whooping cough) with substantial morbidity and mortality is caused by gram-negative pleomorphic bacillus Bordetella pertussis [1]. The highest mortality rate is observed among infants < 6 months of age [2]. In spite of high vaccine coverage, high incidence of pertussis and its mortality rate have been reported from many countries, such as England, Wales, the United States, Portugal, and New Zealand, during the recent years [3]. Pregnant females have low concentrations of pertussis antibodies to transfer and immune their fetuses [4]. Moreover, vaccination against pertussis is licensed for infants after 6 weeks of age while the first dose provides only brief protection [5].

Studies have shown that like maternal tetanus vaccination, other vaccines during pregnancy can protect infants against preventable infections [6]. As pertussis antibodies are efficiently transferred from the mother across the placenta to her fetus [5], vaccination of pregnant mothers with a single dose of tetanus toxoid, diphtheria toxoid, and acellular pertussis (Tdap) between 28 and 38 weeks of gestation, is one of the strategies to control and protect newborn infants against pertussis [3].

Complications related to neonatal pertussis are atypical yet life threatening, including prolonged hospitalization, pneumonia, seizures, encephalopathy, and death in very young infants [1].

Moreover, lower concentrations of pertussis antibodies and insufficient antibodies transport to neonates may increase the risk of pertussis in infants aged < 2 months [7].

### Aims & Objectives

To study the sero prevalence of IgG antibodies to Pertussis Toxin antigen in antenatal women by ELISA.

## MATERIALS & METHODS

This hospital based cross sectional study was conducted among 91 antenatal women attending outpatient department of Osmania Medical College and Hospital, Hyderabad from March 2018 to May 2018. An approval from Institutional Ethics Committee was obtained. Sample size was decided based on convenience and purposive sampling was done to select the study subjects. Antenatal women willing to participate in the study and giving blood sample for the antibody estimation were included in the study.

## METHOD OF DATA COLLECTION

Demographic characteristics of study participants were collected in standard case record form. 5 ml of blood sample was collected from ante cubital vein under all aseptic precautions. Serum samples have been collected and stored at – 20ºC until assayed. Antipertussis specific IgG antibodies (antipertussis toxin) against pertussis were measured by quantitatively enzyme immunoassays using Bordetella pertussis IgG ELISA kit based on sandwich principle. ELISA was performed using IMMUNOLAB Bordetella pertussis toxin IgG ELISA kit. ELISA Procedure: (From IMMUNOLAB ELISA kit) (Fig 1).

Washing Solution: dilute before using as 1+9 with deionized water

**Assay Steps**

1. Prepare a sufficient amount of microtiter wells for the standards, controls and samples as well as for a substrate blank.
2. Pipette 100 µL each of the diluted (1:101) samples and the ready-to-use standards and controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the enclosed foil and incubate for 60 minutes at room temperature.
4. Empty the wells of the plate and add 300 µL of diluted washing solution and it is repeated three times. Rest of the washing buffer is removed by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipetting 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover the plate with the enclosed foil and incubating for 30 minutes at room temperature.
7. Emptying the wells and add 300 µL of diluted washing solution and it is repeated totally three times. Rests of the wash buffer is removed by gentle tapping of the microtiter plate on a tissue cloth.
8. Pipette 100 µL each of the ready-to-use substrate into the wells. Substrate blank is also pipetted.
9. Cover the plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark
10. Terminate the substrate reaction, by pipetting 100 µL each of the ready-to-use stop solution into the wells. Pipette also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm).The cut-off values of less than 20 U/mL was considered negative and 20 -30 was borderline and more than 30 U/mL was positive for antipertussis antibody.

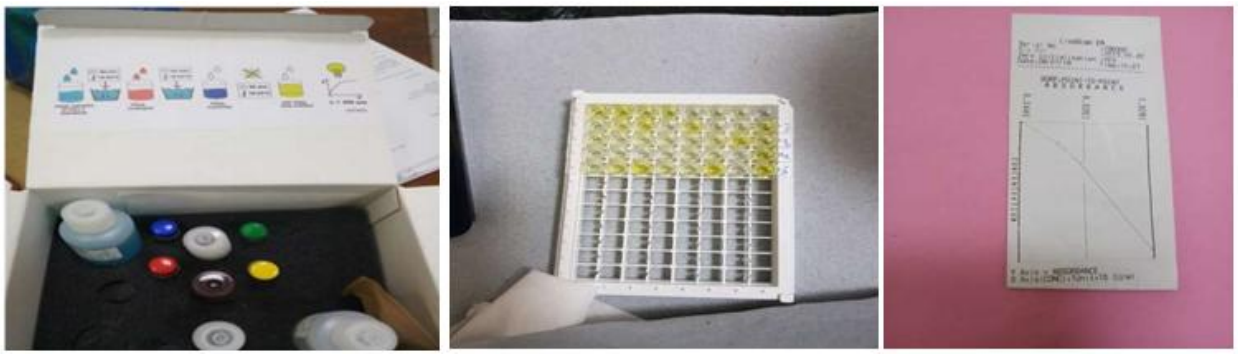

**Fig-1: ELISA kit contents, Microtitre wells, Absorbance graph**

## STATISTICAL ANALYSIS

Data collected was entered in MS-excel and analysed using the same software. Descriptive statistical measures like percentage were applied. Data was presented as graphs

## RESULTS

Antenatal women enrolled in the study were in the age group of 18 – 26 years. All the women were literates. Majority of women responded that they had primary immunisation with DPT in childhood. Frequency of antipertussis antibody was 16.48%, 9.89% & 73.62% respectively among protected, borderline and unprotected antenatal women (chart 1).

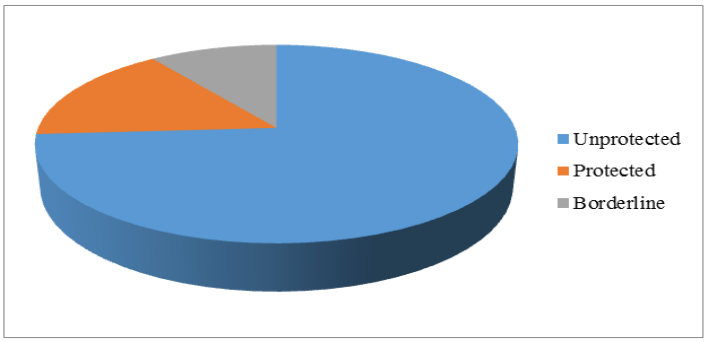


**Chart-1: Frequency of IgG antibodies against Pertussis toxin**

## DISCUSSION

Newborn infants are most at risk as their immune system is functionally immature and they do not acquire protective levels of antibodies until at least 4 months of age (usually after the second dose of pertussis vaccine). The newborn is therefore reliant on IgG antibodies, acquired through passive transplacental transfer, and IgA antibodies, in breast milk, for protection. Studies have shown low levels of maternal pertussis antibodies available for infant protection in the majority of women of childbearing age. Furthermore, the mother has been identified as the main source of pertussis infection to the newborn. Maternal vaccination is a strategy that has the potential to afford protection to the neonate via passive transplacental transfer of maternal antibodies. These antibodies protect infants in the first few months of life, prior to the development of active immunity through routine vaccination [8].

In the prevaccine era, 30 –50% of pregnant women had circulating antibodies against pertussis [9, 10]whereas more recent studies found low anti-PT IgG levels (geometric mean titer, <10 enzyme-linked immunosorbent assay units/mL) in the majority of women[11, 12]. A recent study of maternal IgG antipertussis antibody in U.S. women showed very low levels of antibody in women and their infants to pertussis toxoid, filamentous hemagglutinin and fimbrial proteins and demonstrated that most of this antibody was absent by 2 months of age in infants born to these mothers[13].

Protective immunological memory to B. pertussis is not long-lasting, which is observed after natural infection and immunization with whole cell pertussis vaccines (wP), but is apparently even shorter-lived after use of current acellular pertussis vaccines (aP). Both humoral and cellular adaptive immunity are involved. Low levels of pertussis toxin (Ptx) specific immunoglobulins (Ig) have been shown to correlate with susceptibility to disease. At mucosal surfaces, secretory IgA is the first line of defence against B. pertussis. In serum, (monomeric) IgA is the second most prevalent antibody type after IgG. Serum IgA, which is a poor activator of complement, can trigger effector functions that may extinguish bacteria. IgG, the most abundant antibody type consists of four IgG subclasses: IgG1is the dominant subtype, followed by IgG2, IgG3 and IgG4. These subclasses have distinct effector functions, mediated by differences in the Fc part of the Ig molecule [14].

Babies born to immunized mothers had 2.9 times greater levels of antibodies to pertussis than did control babies [15]. In the present study only 16.48% of women were having protectable levels of antibodies. This indicates that majority of women and newborns born to them were at risk of Pertussis. ACOG (American College of Obstretrics and Gynecologits) and CDC (Centre for Disease Control) also recommends Tdap vaccine to all pregnant women [16, 17].

If a Tdap vaccine in pregnancy is being considered to replace a single dose of TT vaccine it should be assessed for dual coverage for pertussis and tetanus disease. In a small study from Vietnam, vaccination with Tdap in pregnancy resulted in higher cord anti-TT IgG levels compared with vaccination with TT. These results are reassuring that replacing TT with Tdap is not expected to result in inferior immunogenicity against tetanus [18].

Vaccination of pregnant women induces a vaccine-specific immune response to the mothers and to the infants by transfer of vaccine specific antibodies via the placenta and breastmilk which directly protect the infant during the first months of life from the targeted pathogens. The potential of maternal immunization in protecting young infants was made evident by tetanus vaccination during pregnancy which proved reduction in incidence of neonatal tetanus. It is also evident by the decrease in the incidence of severe pertussis disease in young infants in countries that have implemented pertussis immunization programs in pregnancy. During the last decade, an increasing number of countries have included vaccines for pregnant women in their national vaccination programs [18].

In 2011, the US Advisory Committee on Immunization Practices (ACIP) recommended acellular pertussis-containing vaccine (Tdap) to any person, including pregnant women, likely to be in contact with

infants under the age of 12 months. In September 2012, the UK recommended Tdap vaccine for pregnant women ideally between 28 and 38 weeks of pregnancy. In a study by Petousis-Harris H, Walls T, Watson D, et al regarding Tdap Vaccine safety in pregnancy revealed that vaccination with Tdap in pregnancy does not reveal any serious adverse events and it is well tolerated[19].

Immunisation during pregnancy aims to protect infants during the period before they complete their primary immunisations. Antenatal immunisation against tetanus has been recommended for decades and is estimated to have reduced the burden of neonatal tetanus by a large percentage. Recently, many countries like Australia have reported a steep rise in pertussis cases, younger than 2 months of age, who are too young to have been vaccinated and are prone for hospitalisations and sometimes death. Due to this reason, maternal pertussis immunisation has been recommended in many developed countries since 2012.

After birth of an infant, maternal antibodies wane with a half-life of approximately four to six week. Longer duration of protection is associated with higher levels of antibodies. Despite the benefits of maternal immunisation, there are also potential disadvantages. Pre-existing maternal antibodies influence antibody responses following infant immunisation, which is called 'blunting'. Apart from the type of vaccine, blunting of infant antibody production is also influenced by the age of infants at immunisation, the effect being stronger in younger infants. Nevertheless, for acellular pertussis, diphtheria and IPV, the influence of maternal antibodies has been reported to be still evident after booster immunisation at 12 to 24 months of age [20].

Vaccines help in protecting the mother's body from infections and this immunity passes to her baby during pregnancy. This immunity keeps the child safe during the first few months of life until baby gets his own vaccination. Vaccination also protects mothers from getting a serious disease which may affect future pregnancies. Globally, no scientific study exist which shows the risk of fetus after vaccination of pregnant women with inactivated vaccines or bacterial vaccines or toxoids. Benefits of vaccinating pregnant women usually outweigh potential risks when the likelihood of disease exposure is high, when infection would pose a risk to the mother or fetus, and when the vaccine is unlikely to cause harm. Not all vaccinations are safe during pregnancy but some inactivated vaccines are considered safe which can be given to pregnant women who are at risk of infection [21].

IAP ACVIP suggests immunizing pregnant women with a single dose of Tdap during the third timester (27 - 36 weeks gestation) regardless of number of years from prior Td or Tdap vaccination. Tdap has to be repeated in every pregnancy irrespective of the status of previous immunization. An adolescent girl who had received Tdap one year prior to becoming pregnant will have to take it since there is rapid waning of immunity following pertussis immunization. This suggests that Tdap is to be administered during every pregnancy to provide protection to the baby against pertussis [22].

Indian Academy of Pediatrics (IAP) and The Federation of Obstetric and Gynecological Societies of India (FOGSI) recommend Tdap vaccination during adolescent and pregnancy, respectively, the coverage is variable, and TdaP for pregnant women is still not a part of National Immunization Program (NIP)[23].

The incidence of pertussis declined sharply after launch of Universal of Immunization Program (UIP) in India. Prior to UIP, India reported 200,932 cases and 106 deaths in the year 1970 with a mortality rate of <0.001%. During the year 1987, the reported incidence was about 163,000 cases which came down to 40, 508 in 2010 and 39, 091 in 2011 with a decline of about 75%. Maximum cases in 2010 were reported by Andhra Pradesh, Madhya Pradesh, Jharkhand, and West Bengal. However, the reliability and quality of the data is questionable. A large number of cases go unreported, and many non-pertussis cases are reported and clubbed under the head of 'whooping cough' cases. Hence, these figures lack specificity. The actual number may be high considering that the coverage with three doses of DPT vaccine in infancy was 71.5% and only 41.4% children in the age group of 18-23 months had received first DPT booster. The data on pertussis disease and infection in adolescents and adults is sorely lacking and there is no data on Bordetella pertussis infection rates in the community [24].

In a study conducted by Nabaneetha Dash in an article --Antenatal TdaP: It's Time India Adapts, they reported 4 cases of infant pertussis in Christian Medical College, Vellore within a span of 1 week in January 2020. It indicates lack of protection against Pertussis.

In a study in 2018 by Ujjwayini Ray 4 out of 76 infants tested were positive for B. pertussis by Multiplex PCR. Out of them three were not vaccinated and one was vaccinated. Reports of pertussis resurgence and outbreaks are mostly available from the developed world with sophisticated diagnostic facilities and improved surveillance systems of infectious diseases. Pertussis is being underreported from developing countries with large populations. Recently published data from a multinational serosurveillance study of B. pertussis infection among 10–18-year-old Asian children and adolescents included patients from South Korea, Taiwan, China, Sri Lanka, Thailand, Japan, and India showed that there is a significant circulation of B. pertussis among Asian children and adolescents, with one in twenty having serologic evidence of recent infection regardless of vaccination background.

The availability of an effective vaccine against B. pertussis since the 1940s led to a substantial reduction in the morbidity and mortality caused by pertussis worldwide. Under the Expanded Programme of Immunisation (EPI) scheme in India, the pertussis vaccine is administered as a conjugate vaccine commencing from 6 weeks of age, as three doses 4 weeks apart. Young infants below the age of pertussis vaccination are highly vulnerable for contracting the infection. Over 700 million episodes of acute respiratory infections (ARI) and 52 million episodes of pneumonia occur every year in India in children under 5 years of age. ARIs contribute to ~1.9 million childhood deaths per year in developing countries, and 20% of these deaths are in India. Pertussis also contributes significantly to this disease burden [25].

Although Td (tetanus and diphtheria) vaccine has been recently introduced in NIP for adolescent and pregnant mothers to prevent diphtheria outbreaks this studysuggests this is a right time TdaP vaccine should be introduced for antenatal vaccination to prevent infant pertussis [23].

## CONCLUSION

Despite immense progress in reducing the morbidity and mortality of pertussis through universal infant and childhood immunization, it is still a public health problem worldwide. The present study concludes low levels of antibodies in pregnant women to Pertussis. Vaccination is a cost – effective strategy. Lack of awareness and concerns regarding vaccine safety are the common barriers for implementing effective vaccination. Under Universal Immunisation Programme two doses of Tetanus Toxoid have been administered. There is a need for booster dose to be given to pregnant women in the form of TdaP Vaccine to protect the antenatal women and also newborns against Pertussis Diphtheria and Tetanus. One dose of TT may be replaced with TdaP vaccine and this may be helpful for antenatal women and also preterm lowbirth weight babies and Tdap has to be included in Universal Immunisation Programme for the good benefit of pregnant women and also infants.

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