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# High Sero-Prevalence of Dengue IgG Antibodies among Healthy Individuals in Andhra Pradesh, India

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## ABSTRACT

Dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) are increasing in India and Andhra Pradesh. Most cases of DHF and DSS occur at the time of secondary dengue virus infection. It is critical to know, the proportion of population exposed to dengue virus infection; therefore at risk of developing DHF or DSS. We assessed the approximate extent of exposure to Dengue virus infection in Hyderabad, Andhra Pradesh.

A convenience sera sample from 200 apparently healthy individuals attending routine health checkup at a medium-sized urban reference laboratory was collected. The samples were assayed for anti-dengue IgG; in first phase using Dengue IgG Capture ELISA and IgG Indirect ELISA; and in Confirmatory phase using Plaque Reduction Neutralisation Test (PRNT).

Sero-positivity of 10.5 % (21 of 200) with IgG Capture ELISA and 89.5 % (179 of 200) with IgG Indirect ELISA was seen in first phase. 100% sero-positivity was seen in the samples of age ≥ 40 years by Indirect ELISA. In the confirmatory phase, 75% of Indirect ELISA positive samples tested positive with PRNT. Of the 12 randomized blinded samples, all Indirect ELISA negative samples were negative and 5 of the 6 Indirect ELISA positive samples were positive with PRNT. There was a positive correlation between the two assays.

The high sero-positivity dengue IgG of 89.5% indicates that the study population has been exposed to Dengue virus infection. This study warrants the need for further large scale population based studies and identification of circulating serotypes.

***Keywords:*** *Dengue, Seroprevalance, Dengue Indirect ELISA IgG, PRNT, Plaque Reduction Neutralisation Test, Dengue India*

## INTRODUCTION

Dengue fever (DF) is a self-limited illness characterized clinically by fever, frontal headache, retro-ocular pain, muscle and joint pain, and rash, and can be caused by any one of four related but antigenically distinct serotypes of the dengue virus. While the primary infection with dengue fever is not life-threatening, a subsequent or secondary infection with a different serotype can result in dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS), either of which can be fatal. [4] Cases of DF, DHF and DSS are increasing in India[10]. It is documented that cases of DF, DHF and DSS are steadily increasing in Andhra Pradesh[3], from 1 case and no deaths in 2001, to 587 reported cases and 2 deaths in 2007. A report from the World Health Organization in 2006 noted that "current gaps in epidemiological data and surveillance mean that the burden of dengue in India is uncertain."[7] Before 1998 dengue fever had not been reported in

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Tamil Nadu, in that year, 20 febrile patients were tested for anti-dengue antibodies.[6] Seventeen of 20 patients had IgM antibodies to dengue, the remaining 3 patients were IgG positive with thrombocytopenia and pleural effusions. A serosurvey of healthy contacts of the patients found that 95 of 107 (89%) had anti-dengue IgG. It seems likely that a serosurvey of patients' contacts would yield a higher percentage of positive antibodies than a serosurvey of the general population, but the high percentage is still notable. It was also observed that the hospitalized dengue cases and IgG-positive healthy contacts were not geographically clustered but instead were widely scattered, and this could be an indication that dengue fever had spread silently in that area before it was recognized. Active surveillance in Chennai detected a 2.2% prevalence of anti-dengue antibody in 229 schoolchildren (aged 6 to 10, mean 8.02, standard deviation 1.52) in high-risk districts in April of 2001.[2] A second serosurvey of the same children in September 2001, just before the seasonal dengue outbreak of that year, showed that 9.93% of subjects had anti-dengue IgM or IgG. Tuntaprasart also noted a pre-outbreak rise in school children's anti-dengue antibodies, and suggested that the change in schoolchildren's antibody status could serve as a warning system of an epidemic.[9] Experience in Brazil and Thailand has shown that most dengue fever infections are silent or have nonspecific symptoms. Teixeira et al estimated 560,000 new cases of dengue infection (DEN-1, DEN-2, or both) in Salvador, Brazil, over a 12 month period in 1998 and 1999, while only 360 cases were officially reported and dengue transmission was believed to be minimal.[8] Another prospective study from Bangkok[1] compared the incidence of dengue infection in 4 to 16 year old students to their school absences, and found that 87% of newly infected students were asymptomatic or were absent from school for only 1 day, indicating that most cases of primary dengue fever remain unrecognized.

The prevalence of dengue in the general population of Andhra Pradesh has not been assessed. As most cases of DHF and DSS occur at the time of secondary dengue virus infection, it is critical to know what proportion of the population already has been infected with dengue virus and is therefore at risk of developing DHF or DSS. We assessed an approximate extent of exposure to dengue virus by conducting a serosurvey among apparently healthy individuals in Hyderabad, Andhra Pradesh.

## MATERIALS AND METHOD

A convenient sample of 200 apparently healthy individuals presenting to an urban reference laboratory on an outpatient basis for routine health checkup in Hyderabad, Andhra Pradesh, was collected. The following table shows the margins of error for a range of proportions of seropositivity for a sample of 200 subjects:

**Table 1: 200 samples, margin of error for a range of seropositivity proportion**

| Estimate of true proportion | 1% | 5% | 10% | 20% | 30% | 40% | 50% |
|---|---|---|---|---|---|---|---|
| Margin of error | 1.4% | 3% | 4.2% | 5.5% | 6.3% | 6.8% | 6.9% |

The subject's after consent underwent phlebotomy and were assayed for Dengue IgG antibodies. Only individuals between 19 and 70 years of age were included and to optimize immune-competence, history of HIV/AIDS, malignancies, cirrhosis of the liver and renal disease were excluded. Approximately 5 ml blood was collected in a 10 ml Gel / Plain Vacutainer for routine health checkup needs, no additional needle stick was done for the purposes of this study, and no subject underwent phlebotomy solely for the purpose of this study. The subjet's sera was tested in two phases, (i) in the first phase samples were tested using Dengue IgG Capture ELISA (PanBio Australia) and Dengue IgG Indirect ELISA (PanBio Australia). Samples were tested according to the manufacturer's instructions. Results were categorised as negative, equivocal or positive. Equivocal samples were retested and reported as positive or negative as per the test protocol. For IgG Indirect ELISA kit, Panbio Units of > 11 were considered positive and values < 9 as negative. For Dengue IgG Capture ELISA Kit, Panbio Units of >22 were considered positive and values < 18 as negative. (ii) in the second phase or confirmatory phase, the ELISA results were confirmed using Plaque Reduction Neutralisation Test (PRNT). Initially 40 IgG Indirect ELISA positive samples with highest Panbio units were chosen and sent to National Institute of Virology (NIV), Pune, India for confirmation using PRNT. The samples were coded with a separate serial number without revealing the Panbio Units. After this, a second set of 12 samples (6 positives and 6 negative) were chosen randomly, blinded, coded and sent for testing by PRNT. The PRNT assay was carried out in Porcine Stable kidney (PS) cells against DENV-2 (an

Indian isolate 803347), and were tested at three dilutions 1:50, 1:250 and 1:1250 with the titres calculation by Probit analysis.

## RESULTS

Of the 200 individuals who participated in the study, 136 were males and 64 were females. Mean age of the participants was 29 years. 131 (66%) participants were from Hyderabad region and 69 (34%) from other districts of Andhra Pradesh. 21 of 200 (10.5 %) (CI - 6.9, 15.5) were tested positive with Dengue IgG Capture ELISA and 179 of 200 (89.5 %) (CI- 84.4, 93.0) were tested positive with IgG Indirect ELISA. 20 of the 21 IgG capture ELISA positive were positive for indirect ELISA and 20 of the 21 Indirect ELISA negative were tested negative with Capture ELISA. Indirect ELISA seropositivity increased with age and all subjects aged 40 years and above had positive result. No difference was found in seropositivity based on geographical location and gender.

Of the 40 Indirect ELISA positive samples tested using PRNT, 30 were positive (titres >50), 9 were negative and one sample could not be tested (insufficient sample). There was no correlation between Panbio Units and PRNT titres. In the next set of 12 blinded samples, all 6 ELISA negative samples were tested negative with PRNT, but among the 6 ELISA positive samples, 5 tested positive with PRNT. There was a significant correlation between the PRNT and Ig G Indirect ELISA assays (p-value 0.015) (Table 2). Univriate and multivariate analysis for factors gender, region and age group with seropositivity with no significant association between them. (Table 3)

**Table 2: Comparision of Results of Indirect ELISA and PRNT of the blinded samples.**

| | | IgG Indirect ELISA Result | | Total |
|---|---|---|---|---|
| | | Negative | Positive | |
| PRNT Result | Negative | 6 | 1 | 7 |
| | Positive | 0 | 5 | 5 |
| Total | | 6 | 6 | 12 |

**Table 3: Univariate analysis of factors associated with seropositivity.**

| Covariates | n tested (%) | n positive(%) | Odds ratio (crude) | 95% Confidence | *P*-value |
|---|---|---|---|---|---|
| Males | 136 (68%) | 121 (89%) | 1 | | |
| Females | 64 (32%) | 58 (91%) | 1.2 | (0.4-3.2) | 0.722 |
| Hyderabad | 131 (66%) | 116 (89%) | 1 | | |
| Other districts | 69 (34%) | 63 (91%) | 1.4 | (0.5-3.6) | 0.547 |
| <25years | 82 (41%) | 68 (83%) | 1 | | |
| 26-35 years | 87 (43%) | 81 (93%) | 2.8 | (1.0-7.6) | 0.047 |
| 36-45 years | 18 (9%) | 17 (94%) | 3.5 | (0.4-28.5) | 0.242 |
| >45 years | 13 (7%) | 13 (100%) | N.A | | |

## CONCLUSION & DISCUSSION

Based on the findings it could be concluded that the local population is largely exposed to the Dengue virus. The study warrants the need for further large scale population based studies and identification of circulating serotypes.

During the past 50 years, several outbreaks were reported in different parts of the country. However there have been no reports to our knowledge describing the magnitude of exposure to virus in healthy population of the region. Although the data from National Vector Borne Disease Control Program (NVBDCP) shows that the number of dengue cases and deaths in the state is low, we feel that the actual number of dengue cases are grossly underestimated. The NVBDCP activities mainly serve the rural areas, with little coverage of the urban areas. Cases from private hospitals and nursing homes are not included in the surveillance as there is no strict mandate for private hospitals to report dengue cases in India. According to HMRI (Health management and research institute) monograph 12,528 dengue positive cases were recorded from all the 23 districts of Andhra Pradesh from April 2008 till January 2011 using rapid tests and ELISA tests, based on data from 2700 registered labs from the state.[13] Unpublished data from a tertiary hospital in Hyderabad, recorded had more than 400 admissions from August 2009 and November 2009.

To the best of our knowledge, this is one of the first studies in the Hyderabad region for dengue seroprevalance. High seropositivity noted in this study indicates that this population has been largely exposed to Dengue virus infection. Our results correlated well with the previous studies from Delhi and Tamil Nadu showing a seropositivity of 89% [14] and 77.6% [15] respectively. Studies from around the world reported a percentage seropositivity of 100%, 98% and 91% in Jamaica, Dominican Republic and Brazil respectively.[16,17&18] The increase in percentage seropositivity with age and having almost equal percentage of seropositivity among males and females is in agreement with the study from Dominican Republic.[17] Studies have shown that Dengue outbreaks are associated with a switch in circulating Dengue serotype.[19] Therefore identification of circulating serotypes and monitoring their switching patterns can serve as an early warning system for a possible Dengue outbreak enabling health system to respond effectively. This study emphasises the need for large population based studies to estimate the seroprevalence of dengue in conjunction with serotype identification.

For Sero-epidemiological purposes, IgG Indirect ELISA kits should be used to identify past primary infections and correlates well with the previously used Hemagglutination Inhibition assay (HI) and has largely replaced it.[20] Commercial ELISA kits can detect all four serotypes of Dengue and can also detect cross-reacting antibodies to other Flaviviruses like Japanese Encephalitis (JE) and Yellow Fever (YF) virus. Andhra Pradesh has low incidence of JE[21] and YF is nonexistent. So the possibility of detecting false positives in our study from cross-reacting antibodies is less.

### Limitations of the study

As the study was not randomised and population based, recruitment bias is possible. However, this serosurvey provides preliminary estimates of Dengue IgG antibodies among the population. As the PRNT was performed against only DENV 2, there is a possibility of getting a higher number of PRNT positivity if other serotypes were included in the testing.

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