Dengue Sero-surveillance in Delhi: A Community-Based Study

Shantanu Sharma¹, VK Gupta², Anita Chakravarty³, Suneela Garg⁴

Abstract

**Background:** IgG ELISA is useful as an epidemiological tool when used to establish sero-prevalence. Extensive background information is required before selecting sites for dengue vaccine trials like types of dengue virus that are causing disease, preferably over a number of years, sero-prevalence of dengue IgG antibodies, prevalence of each serotype, rates of infection, specific mortality, etc.

**Aims and Objectives**
- To study the prevalence of dengue IgG antibodies in the serum of adults in urban slums of Delhi
- To study the knowledge, attitude and the practice of study subjects about the prevention of mosquito breeding and dengue fever

**Methodology:** This cross-sectional study was conducted from January 2013 to December 2013 in two urban slums of Delhi, Balmiki Basti and Delhi Gate. All adults aged 20 years and above were included in the study irrespective of sex and previous history of dengue. Knowledge, attitude and practices against mosquito breeding were also taken through predesigned, pretested questionnaires.

**Results:** A total of 49(20.5%) adults from Balmiki Basti and 190 from Delhi Gate were enrolled in the study. Mean IgG dengue OD levels of the study subjects was 1.62±0.568. Of the total, 86% were positive for dengue IgG positivity while 13% negative and 1% equivocal. Of all participants, 214(89.9%) were aware of mode of dengue transmission while 25(10.1%) were not. Majority of the participants 137(57.3%) used liquid vaporizers for prevention of mosquito bites.

**Discussion:** The current study reported that 85.4% of the healthy population in urban slums of Delhi is seropositive for dengue IgG, which indicates the unprecedented extent of exposure to dengue virus. It is 7.8% higher than the study conducted in Delhi in 1998(77.6%). It coincides with the rising trend of dengue cases every year in Delhi, making it an endemic region now.

**Keywords:** Dengue, Sero-prevalence, IgG antibody, Sero-epidemiology

Introduction

Dengue has become one of the most important vector borne diseases over the decades with a steady rise of global incidence, increasing geographic distribution and a transition from epidemic transmission with long inter-epidemic intervals to endemic with seasonal fluctuation.¹ WHO currently estimates there may be 50–100 million dengue infections every

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A total of 239 samples were conducted for a period of 12 months from January 2013 to December 2013. The samples were taken in the inter-epidemic period of dengue. It was carried out in Delhi and two urban areas of central Delhi, which include Balmiki Basti (urban slum located near Ferozeshah Kotla in the central district of Delhi) and Delhi Gate (urban slum in the central district of Delhi). All males and females aged 20–60 years residing in the above-mentioned areas constituted the study population. At 95% confidence level and taking the prevalence of sero-positivity of dengue IgG in Delhi population to be 30% and with a relative error of 20%, the sample size came out to be n=225. A pre-tested, semi-structured questionnaire was used containing items on identification data, past history of diagnosed dengue virus infection, risk factors regarding mosquito breeding, spread of dengue infection, knowledge, attitude and practice of people regarding prevention of mosquito breeding. The questionnaire was based on the validated questionnaire taken from previous studies. A total of 239 samples were taken from the two areas mentioned above. The total population of Delhi Gate was 7745 and that of Balmiki Basti 1026. The number of adults taken from Delhi Gate was 204 and from Balmiki Basti 35. There are 1434 households in Delhi Gate and 213 in Balmiki Basti. 100 households were taken from Delhi Gate and 25 from Balmiki Basti according to an average two adults per household. The houses were selected systematically. Every 14th household was selected from Delhi Gate and every 10th from Balmiki Basti. The adults were provided the description of the project, an informed consent form and questionnaires. A unique identification number was given to each subject enrolled in the study and was recorded in all study documents (informed consent form, data collection tools, blood sample, sera samples). Subject number comprised of five numeric digits: First two digits corresponded to the study site and the other three digits to the chronological order of enrollment for the study site. For the site “Balmiki Basti,” code was 01 and for “Delhi Gate” 02. For instance, the first subject enrolled in Balmiki Basti center was identified with subject number =01001, and the fifth subject at Delhi Gate center=02005.

Information regarding the reasons why some subjects refused to participate in the study, upon proposal by us, was documented in a form called “refusal log”. The geographic position reference and reason of refusal was recorded. The recording of this information was done to assess the representativeness of the study population sample. No code was assigned to people refusing participation. Each vacutainer tube was identified by a label with the subject identification number and the date of sampling. Samples collected were recorded on a sample identification list which was then taken to the laboratory for further processing.

The subjects were informed that they had the right to...
withdraw themselves from the study at any time. On refusal, they were asked for the reason. A total of 16 subjects refused to participate. The most common reason was fear of needle used for collection of sample (62.5%). Fifteen percent of the participants replied that they were not interested in the study while 22.5% never had dengue, so no perceived need of participating in the study and the fact that they already had blood investigation done. Venous sample of about 5 mL was taken from each subject. Every study subject was also explained about the ways to prevent the breeding of mosquitoes, the need to keep the surroundings clean, prevent the artificial collection of water in empty vessels, tires, old and unused utensils, to spray the oil in coolers or regularly changing water.

Sample processing was done according to ELISA kit manufacturer’s* guidelines in the Department of Microbiology, Maulana Azad Medical College, New Delhi. The diagnostic dengue ELISA IgG used in the study is an indirect ELISA intended for the detection of antibodies to DV types 1–4. This assay is specific for IgG and uses a horse radish peroxidase conjugate with 3,3',5,5' tetramethylbenzidine (TMB) as substrate. Each antigen-coated well contains equal proportions of inactivated, purified dengue virus types 1–4.

The data was entered in MS-Excel and analyzed by using SPSS software version 17. Qualitative data was expressed in percentages with 95% confidence interval. Quantitative data was expressed in mean±SD. Chi-square test/Fisher’s Exact test was used for qualitative variables. Cross tabulation was done to assess the relationship between dependent and independent variables. “P” value <0.05 was considered significant.

Ethical Considerations

The objective and procedure of the study was explained to the participants. Written informed consent was taken from the study subjects. The option to opt out of the study was kept open without any clause. The data was kept confidential and was used for research purpose only. The participants were given health education about mosquito-borne diseases and use of personal protective measures. The study was approved by institutional ethical committee.

Results

Socio-demographic Characteristics of the Study Population

A total of 49(20.5%) adults from Balmiki Basti and 190 from Delhi Gate were enrolled in the study. There were 82 males and 157 females from the two areas. The two most common communities in the study areas were Hindu (59.4%) followed by Muslim community (40.6 %). Among Hindus, a few were Sikhs (1.5%) and Jains (1.1%). Literacy rate in Balmiki Basti was 83% which was higher than that in Delhi Gate (75.2%).

Young adults constituted major chunk (35.5%) of the total study population whereas least representation was by adults of 50–60 years. Average number of people with monthly family income Rs. 8707 or greater were more in Delhi Gate area compared to Balmiki Basti. Mean age of study participants was 37±14.3 years (mean±SD) in the two areas.

The mean age of males (37 years±12.75) and females (37 years±15.2)(independent ‘t’ test; p=0.862) of the study population was approximately similar. Only 4 of the total 239 subjects had had dengue in the past. Mean IgG dengue OD levels of the study subjects are given in Table 2. Frequency distribution of prevalence of dengue IgG positivity is shown as a pie chart in Fig. 1. Dengue positivity among adults in the study population was 86% while 13% were negative for it.

Table 1.Index Values for Dengue IgG Antibodies

| Index  | Result  | Interpretation                                                                 |
|--------|---------|--------------------------------------------------------------------------------|
| <0.9   | Negative| No evidence of a past dengue infection. If a recent dengue infection is suspected, this can be confirmed by testing a further specimen 10-14 days later |
| 0.9–1.1| Equivocal| Suggest samples should be re-tested. If specimen remains equivocal following repeat testing then the specimen may be tested by an alternate method or another patient specimen obtained and tested. |
| >1.1   | Positive| Presence of detectable IgG antibodies indicates evidence of past or recent dengue infection. In areas where multiple flaviviruses co-circulate the presence of cross-reactive flaviviruses antibodies should be considered. |

The data was entered in MS-Excel and analyzed by using SPSS software version 17. Qualitative data was expressed in percentages with 95% confidence interval. Quantitative data was expressed in mean±SD. Chi-square test/Fisher’s Exact test was used for qualitative variables. Cross tabulation was done to assess the relationship between dependent and independent variables. “P” value <0.05 was considered significant.

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Table 2.Mean IgG OD Levels of Dengue

| Dengue OD Value |  |
|-----------------|--|
| Mean±SD         | 1.62±0.568 |
| Median          | 1.84 |
| Range           | 0.085-2.087 |
Figure 1. Pie Chart Showing Distribution of Prevalence of Dengue IgG Positivity

Figure 2 depicts age-wise distribution of dengue IgG positivity. Maximum percentage of negatives for dengue IgG antibodies was in 20–29 years old age category while least in 50–60 years. Dengue positivity is increasing with age except for a dip in 40–49 years old category as shown in Table 3.

![Dengue IgG positivity](image)

Figure 2. Bar Chart Depicting the Trend of Dengue IgG Positivity over the Age Categories

Note: All figures are in percentage; x axis=age groups; y axis=percentage

Table 3. Age-Wise Mean Dengue IgG OD Values with SD

| Age Categories (in Years) | Mean±SD of Dengue OD |
|--------------------------|----------------------|
| 20–29                    | 1.469±0.695          |
| 30–39                    | 1.70±0.4729          |
| 40–49                    | 1.589±0.604          |
| 50–60                    | 1.796±0.3133          |

Univariate analysis was done to find any association between positivity of dengue IgG and variables like age, sex, study center, anemia and religion in Table 4. Males were proportionately more positive for dengue IgG antibodies (93.9%) compared to females. Sero-prevalence for dengue IgG was higher in Delhi Gate (15.3%). As per the study, none of the practices against dengue affected dengue IgG positivity. No statistical significant association of dengue IgG positivity with use of any personal protective measures (PPM) (p value=0.501) or with frequency of use of PPM (p value=0.295) or with time of use of PPM (p value=0.863) was found.
Table 4. Association of Dengue Antibody Positivity with Different Variables

| Variable* | Dengue IgG Positivity | Total 239 | P value, χ², df |
|-----------|-----------------------|-----------|----------------|
|           | Negative (N=32) N (%) | Equivocal (N=3) N (%) | Positive (N=204) N (%) |
| Sex       | Male                  | 5(6.1)    | 77(93.9)       | 82 | 0.022, 7.592, 2 |
|           | Female                | 27(17.2)  | 127(80.9)      | 157|
| Age(in years) | 20–29 | 19(22.4) | 1(1.2) | 65(76.5) | 85 | 0.049, 12.658, 6 |
|           | 30–39                | 5(9.3)    | 1(1.9) | 48(88.9) | 54|
|           | 40–49                | 6(15)     | 0      | 34(85)   | 40|
|           | 50–60                | 2(3.3)    | 1(1.7)  | 57(95)   | 60|
| Center    | Balmiki Basti        | 3(6.1)    | 1(2)    | 45(91.8) | 49 | 0.219, 3.037, 2 |
|           | Delhi Gate           | 29(15.3)  | 2(1.1)  | 159(83.7)| 190|
| Religion  | Hindu                | 19(13.4)  | 1(0.7)  | 122(85.9)| 142| 0.651, 0.859, 2 |
|           | Muslim               | 13(13.4)  | 2(2.1)  | 82(84.5) | 97|
| Is dengue preventable | Yes      | 14(10.1) | 2(1.4)  | 123(88.5)| 139 | 0.364, 4.32, 4 |
|           | No                   | 3(12.8)   | 0       | 21(87.5) | 24|
|           | Don’t know           | 15(19.7)  | 1(1.3)  | 60(85.4) | 76|
| Time of use of repellant | Day | 1(7.7)     | 0       | 12(92.3) | 13 | 0.863, 2.54, 6 |
|           | Night                | 25(14.5)  | 3(1.7)  | 145(83.8)| 173|
|           | Both day and night   | 4(14.8)   | 0       | 23(85.2) | 27|
|           | Don’t use            | 2(7.7)    | 0       | 24(92.3) | 27|
| Using any PPM | Yes | 30(14.2)   | 3(1.4)  | 179(84.4)| 212 | 0.50, 1.38, 2 |
|           | No                   | 2(7.4)    | 0       | 25(92.6) | 27|
| Family monthly income(Rs.) | 1744–5223 | 3(20) | 0 | 12(80) | 15 | 0.026, 14.36, 6 |
|           | 5224–8706            | 10(16.4)  | 0       | 51(83.6) | 61|
|           | >8707                | 16(18)    | 0       | 73(82)   | 89|
|           |                     | 3(4.1)    | 3(4.1)  | 68(91.9) | 74|

*Factors are not mutually exclusive

No statistical significant association of occupational status (p=0.711) and educational status (p=0.442) with dengue IgG positivity was found.

Table 5. Sero-positivity of Mean Japanese Encephalitis and Mean Dengue Antibody Levels with Variables

| Variables* | Mean Dengue OD Value** | P Value |
|------------|------------------------|---------|
| Study site | Balmiki Basti | 1.76±0.453 | 0.05 |
|           | Delhi Gate | 1.58±0.58  |  |
| Sex       | Male | 1.75±0.42   | 0.011 |
|           | Female | 1.55±0.621 |  |
| Religion  | Hindu | 1.64±0.57  | 0.473 |
|           | Muslim | 1.59±0.55  |  |
| Usage of any PPM | Yes | 1.61±0.584 | 0.318 |
|           | No | 1.72±0.410  |  |
| Is dengue preventable? | Yes | 1.68±0.531 | 0.098 |
|           | No | 1.65±0.59   |  |
| Time of using repellent | Only day | 1.75±0.51 | 0.617 |
|           | Only night | 1.6±0.58  |  |
|           | Day and night | 1.57±0.628 |  |
|           | Don’t use | 1.72±0.417 |  |

*Factors are not mutually exclusive

**All values are expressed as mean±SD
Knowledge and Attitude about Dengue

Of all participants, 214(89.9%) were aware of mode of dengue transmission while 25(10.1%) were not. Among those who were aware of modes of transmission of dengue, 206(96.2%) correctly told it to be the mosquito, whereas a few others mentioned water, food or water and mosquito combined. Out of 220 participants who responded yes that they knew the places of mosquito breeding, 157(71.36%) told it the stagnant water only, 52(23.63%) answered clean and stored water while others 11(5%) told it to be the stagnant water along with coconut shells, open tanks and unused utensils. Majority of the participants 189(86.0%) answered rainy season as the most frequent season for dengue transmission while 26(12%) said summer and two participants each told autumn and spring. Regarding ways of prevention of mosquito breeding, one-fourth of them told keeping hygiene alone is the method for prevention, three-fifths replied various combinations of methods like preventing water accumulation, changing water in coolers regularly, discarding old and unused utensils while only 5% told all methods. Slightly less than one-fourth (23.4%) of the participants were not aware of any symptom of dengue whereas 106(45%) answered correctly all the major symptoms while 77(32.2%) answered it only fever or fever with rashes. Most common media form where participants had got to know about dengue was television or radio(66%) followed by newspaper(24%). More than 50% of the participants knew that dengue is preventable.

Practices against Dengue

Majority of the participants 137(57.3%) used liquid vaporizers. While only 11(4.6%) subjects used mosquito creams, 48(20%) also used sprays. But 27(11.3%) of them did not use any PPM. Only 27(12.7%) participants used PPM both during day and night while majority 173(81.6%) used it only during night. In 24.3% subjects' houses, mosquitoes were found in and around home. On inspection, coolers were found dry in 95.8% of the houses but in 15.1% of the subjects stagnant water was found in and around their houses which could be the potential breeding sites. Slightly above 50% use PPM daily and change or replace them as they get finished while other change them once weekly or use them when they find excessive mosquitoes. No statistical significant association was found between family income and frequency of use of PPM whether daily or once or twice a week or when felt mosquitoes (p value=0.654), and not with the knowledge of participants whether dengue was preventable or not (p value=0.468). There was statistical significant association between time of use of PPM and family income implying that families with higher income use PPM during both day and night (p value=0.022). With increase in family income, the percentage of families using any PPM increased but the association was not statistically significant (p=0.55).

Sero-prevalence of Dengue IgG Antibodies

The current study reported that 85.4% of the healthy population in urban slums of Delhi was sero-positive for dengue IgG which indicated the unprecedented extent of exposure to dengue virus. Large numbers of individuals with IgG positivity would be at alarming risk of developing dengue hemorrhagic fever (DHF) with introduction of other serotypes owing to cross reactivity. In 2010, sero-prevalence of dengue IgG in American Samoa was found to be 95.6% (CI: 93.9%–96.8%). On the other hand, it was 31.3% in central part of India and 67.2% in Lahore, Pakistan. Similarly, Sultana et al. found that 42.9% of suspected cases of dengue fever in Chittagong, Bangladesh, had anti-dengue IgG in their serums. In a study by Kurukumbhi et al. in 1998 in Delhi in adults, the sero-prevalence was 77.6%. Differences in disease frequency can be explained on the basis of level of urbanization and deterioration of environment. In many parts of the world, dengue infection is predominantly a childhood disease; however, it affects adult population primarily during first few years of its emergence. Results of the current study also confirmed this notion. Adults aged 30 and above showed higher IgG positivity, and an upward trend was observed with increasing age. Similar findings were also reported by Ukey et al. in India, where most affected population stratum was in age range of 15–30 years and that of Sri Lankans was between in the age range of 26–40 years. The present study showed that the prevalence of dengue antibodies among adults in urban slums of Delhi, increases with age, indicating high, relatively stable, transmission rates over many years. Other studies in dengue endemic areas with similar sero-prevalence, also showed that antibody prevalence increases with age.
Delhi were infected more than female population, yet this difference was not observed in other studies elsewhere. Level of awareness about dengue signs and symptoms and protective measures was found unsatisfactory among the study population, yet no statistical association was observed between anti-dengue IgG sero-positivity and knowledge (p value=0.864) about disease transmission, potential breeding sites, complications, and preventive measures or with education status (p value=0.442) and occupational status (p value=0.711). There were obvious differences in sero-prevalence among studied individuals based on their monthly income implying greater dengue positivity among individuals with higher family income (p value=0.026). These findings were contrary to the studies by Duncombe at al. and Amarasinghe et al., where it was reported that dengue is related to poor socioeconomic conditions and poor knowledge. Similarly, Braga et al. in Brazil found an inverse relationship between socioeconomic status and sero-prevalence. Magnitude of dengue burden was higher (91.1%) in socioeconomically deprived areas compared to intermediate (87.4%) and high-status regions (74.3%). The results of this study should be interpreted in light of the consideration that study participants were limited to adult healthy population, excluding children for not granting ethical approval and using relatively small sample size for prevalence estimation; however, results are significant enough and provide evidence in order to devise a surveillance system for detecting dengue outbreaks in an early stage for preventive actions. Incidence rate calculated in the present study was 52%, which is more than four times of that reported by a study in Southern Vietnam by Thai et al. in 2005 (11.7%). The reason for such a high incidence rate could be endemicity of dengue in Delhi or could be the urban slums in Delhi, which are the niche for dengue mosquito breeding due to unhygienicity, water collection and low usage of PPM.

Although the indirect IgG-ELISA used is a suitable tool for detecting dengue IgG antibodies and has shown high sensitivity and specificity, cross reactivity may occur with other flavivirus antibodies. For India, elimination of dengue fever, dengue hemorrhagic fever, dengue shock syndrome, is probably best achieved via mass dengue immunization programs when vaccines become available.

DHF is emerging as a major public health problem, hence effective prevention and control programs are required. These programs depend on improved surveillance designed to provide early warning of dengue epidemic. The sero-prevalence of dengue IgG antibodies has increased over the years in Delhi which puts residents at risk for dengue hemorrhagic fever with other serotypes. So, the development of a vaccine and implementation of other preventive measures for mosquito control is the need of the hour.

Dengue IgG positivity is significantly associated with sex, age, and family income. This implies elderly people are more susceptible to severe forms of dengue fever due to other serotypes against which they lack antibodies in their blood. Information, education and counseling activities for educating them to use personal protective equipment for preventing them from mosquito bites and also follow vector breeding prevention techniques needs to be conducted.

Knowledge Attitude and Practices against Dengue

The results of this study were comparable to that conducted in 2001 by Van Benthem BH et al. in Thailand in which only 67.0% subjects had heard about dengue. Of these, 98.0% knew that dengue is transmitted via mosquitoes and 74.0% mentioned that dengue vector bite during daytime. In a cross-sectional survey in 2003 by Matta et al. in Safdarjang OPD patients, New Delhi, only 82.4% respondents knew that dengue fever is transmitted by mosquitoes. Only 44.5% subjects enumerated options like checking coolers, tires, flower pots to prevent mosquito breeding. 61.8% persons could enumerate one symptom. Regarding knowledge about breeding, 79.8% respondents knew about breeding places of mosquitoes. It is concluded that though the knowledge regarding dengue is good in the general population, practice of checking coolers, tires and flower pots is quite poor. On the contrary, a study from Kuala Kangsar concluded a significant association between knowledge of dengue and attitude towards Aedes control.

Use of Personal Protective Measures against Mosquitoes

A total of 88.7% subjects were using any one of the PPM. These figures are higher as compared to what was mentioned in another study done in Kerala, which found that most (80.0%) of the rural and all of the urban households reported using at least one personal protective measure against mosquitoes. The results are also higher than what was found by another study which revealed that 84.2% of study subjects were using any one of the available PPMs against mosquito-borne diseases. The use of mosquito-repellent coil and liquid vaporizers was commonest among all methods by 22.6% and 58.98% occasions respectively. No association was seen with gender as seen in previous studies, where the practices of PPM against mosquitoes was almost equal in females as compared to male respondents. Significant association was found between economic status and time of use of PPM against mosquitoes in the present study, implying that the families with higher income use PPM during both day and night (p value=0.22). Some of the previous studies like one conducted by Syed et al. have also shown an association between income and use of PPM.

Use of PPM was more among higher-education classes as compared to lower-education classes. This is similar to the
findings of another study done by Pandit et al., where commercial products like coils and mats were used more among literate households compared to illiterate families (odds ratio=2.32). About frequency of usage of PPM, it was found that 58.1% people were using PPM daily. The findings can be compared with another study conducted in Pondicherry, where 48% in urban areas reported using PPM daily and 47.0% in urban areas were using PPM seasonally. Only 6.1% subjects were using PPM in daytime. This signifies the importance of using PPM in day time also because dengue vector bites mainly in daytime.

Limitations of the Study
As this study was done in Delhi, results may not be extrapolated to all areas in different parts of the country because of difference in urbanization process. Since only IgG antibody was tested and not IgM simultaneously, the sero-positivity of dengue could be higher than actual due to false positives as a result of cross reactivity. Children’s IgG status is a better tool for sero-epidemiology compared to adults’ IgG status, who were not included in the study. Since the sample size was small, different sociodemographic parameters which did not come significant for any association with dengue or Japanese encephalitis antibody status might have turned positive and would have generated a new hypothesis.

Conflict of Interest: None

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