CME LIVE OPEN ACCESS

Journal of Biomedical and Engineering Research

Research Article

Sero Prevalance of Dengue Cases, a Study at a Tertiary Care Hospital in Chhotaudepur, a Tribal District of Gujarat

Sajad ahmad Malik*, Thakur Arti, shugufta shakeel, Prasad Satish and Kaur Balwinder

Assistant Professor at Bhai gurdas group of institutions sangrur Punjab India.

Corresponding Author: Sajad Ahmad Malik, Assistant Professor at Bhai gurdas group of institutions sangrur Punjab India.

Received: 2023 Aug 25 **Accepted:** 2023 Sep 14 **Published:** 2023 Sep 19

Abstract

Background: Dengue fever is one of the most important mosquito borne arboviral disease and is a major international public health concern. Disease is mainly found to affecting the tropical and Subtropical countries of the world.

Aims & Objective: This study was conducted to assess the Prevalence of dengue virus infection in clinically suspected cases coming at tertiary care Hospital in Chhotaudepur one of the tribal district of the Gujarat, state.

Material and Methods: The study was conducted during the months from June 2022 to December 2022. Total 336 serum Samples were collected at the Laboratory Department of General Hospital from the patients Suspected for dengue cases. 3-5 ml of blood sample was collected in plane vacuette and tested for dengue NS1 antigen and IgM antibody by ELISA method. The results data were analyzed for Socieo-demographic features, seasonal variations and statistically to found their significance.

Results: Out of total 336 dengue suspected cases, 39 were found positive for Dengue NS1Ag That's why the seroprevalance of dengue NS1 positive cases was 11.61% (n=39). Among 13.57% Cases (n= 22) were male and 6.55% cases were of females (n= 17). Among the seasonal trend the Peak of dengue cases were found in the month of October (18 out of 105) with gradual increase, whereas the lowest numbers of dengue cases was detected in the month of June and December. Among the total 20 dengue IgM test only 3 were found positive for dengue infection.

Conclusion: The most of the dengue cases were found to be belonged from the younger age group. Dengue Cases appear slowly and reach to the peak in the post monsoon season which coincide with the Increased vector breeding with favorable seasonal habitat. With respect to epidemic measure to detect and control early infection, the dengue NS1 antigen ELISA test appear as an index Diagnostic test in term of its rapidity, ease of performance, and cost effectiveness.

Keywords: Dengue; Dengue Early ELISA; IgM Antibody Capture Enzyme Linked Immunosorbent Assay; Real Time Reverse Transcriptase Polymerase Chain Reaction; Vector

1. Introduction

Dengue is the most common disease of tropical and subtropical area and also called as "tropical flu". It is a viral disease transmitted to humans by the bite of female Aedes mosquitoes carrying dengue virus [1]. Heavy rainfall favor vector breeding results the spreading of the disease, resulting many outbreak or epidemic. In 2022 WHO reported that Incidence of Dengue infection has increased dramatically worldwide in recent decades, from 505 430 cases in 2000 to 5.2 million in 2019 (WHO). The dengue virus is a member of the genus Flavivirus and family Flaviviridae. There are the four serotypes of dengue virus (serotypes DEN1- DEN4) and all four serotypes have similar natural history, where the humans act as the primary host and Aedes mosquitoes of the subgenus Stegomyia (especially Ae. aegypti, Ae. albopictus and Ae. polynesiensis) act as the secondary mosquito vectors [2]. Laboratory diagnosis of dengue provided the confirmation of dengue infection. Thereby Laboratory diagnosis plays a

key role for the epidemiological surveillance to control and prevent the spread of disease [3]. Dengue NS1 antigen is detectable from day 1 of fever to 5 days of fever in both primary and secondary infections. NS1 antigen is shown to be highly specific viral marker making it extremely reliable parameter for diagnosis of dengue infection from day 1 of fever [4]. And so can detect the earlier infection. One modeling estimate indicates 390 million dengue virus infections per year of which 96 million manifest clinically [5]. In 2021 Naik et al; had demonstrated that Ahmedabad district (57%) had a high percentage of infection among the four district [6].

Ahmadabad, Vadodara, Jamnagar, and Anand of Gujarat. However there is no record about the Study of seroprevalence of dengue infection in triable area of District Chhotaudepur though triable people are mostly malnutritious and immune-deficient due to their economic status, Poverty leading to more prone toward sever dengue infection. The effective control and Preventive measure programs can be implemented after getting the data of the sero-prevalence of Dengue infection. Thereby this study was done to assess the prevalence of dengue virus infection in clinically suspected patients coming at tertiary care Hospital in Chhotaudepur, one of the tribal District of Gujarat.

2. Materials and Methods

This study was carried out from month of June 2022 to December 2022. Total 336 serum samples were collected from the Laboratory Department of General Hospital, "Chhotaudepur" Gujarat. The patients having fever ≤ 5 were tested for (1) non-structural (NS1) antigen, by ELISA kit (Oscar Medicare Pvt. Ltd.) and those with fever ≥ 5 days were tested by dengue IgM ELISA capture test kit supplied by National Institute of Virology (NIV), Pune under the NVBDCP [7].

Serum separation:- The blood specimen was collected in serum separator red toped tube. 200 µL serum sample was transferred to the properly labeled eppendorf tube and stored at 2-8oC up to 7 days to test in a batch.

Enzyme linked Immunosorbent Assay (ELISA):- The serum samples were tested for dengue NS1 antigen by ELISA test as per the instructions provided in the kit. Interpretation of the result was done by observing optical density obtained in the ELISA reader.

Dengue NS1 Ag ELISA test:- All reagents of the kits were equilibrated to room temperature (20-25˚C). Required numbers of Micro wells strips were inserted into the strip holder. Plate map data sheet was then prepared where five micro wells were required for controls: A1 (blank), B1 and C1 (negative control) and D1, E1 (positive controls).

50µl of sample dilution buffer was added in each well except A1. 100µl controls and samples were added in the designated well with proper mixing. Strips were sealed with plate sealer and incubated for 60 minutes at 370C. After incubation, all wells were washed five times with diluted wash buffer by filling and aspirating approximately 350µl diluted wash buffer and then wells were blot dried. 100µl diluted conjugate was added in each well except A1 and incubated for 30 minutes at 370C. After incubation, wells were again washed five times. 100µl substrate was added in each well including A1 in dark and incubated at room temperature in dark for 15 minutes. 100 µl stop solution in all wells including A1 and optical density (OD) was taken in ELISA reader machine (at 450 nm with 630 nm wavelength).

Run Criteria:- The individual absorbance value of negative control should be less than 0.1. and of positive control should be more than 1.0.

Interpretation of the Result: - If the test passed the run criteria then further cut off value was calculated by applying following formulae:
Cut off = 0.2 + Average of NC

The sample with absorbance value ≤ cutoff value was considered as non reactive. Whereas the sample with absorbance > cutoff value was considered as reactive.

Anti-dengue IgM-Capture ELISA:- IgM-capture ELISA was performed according to standard protocol provide with kit by NIV, Pune.

Preparation of working reagents:- Serum samples were pre-diluted in the 1:100 ratio. The anti-Dengue HRP conjugate was diluted with diluent in 1:1 ratio, gently mixed and left at room temperature (20-25˚C) for 60 minutes. Preparation of TMB Substrate: 5 ml of each TMB substrate A and TMB substrate B was mixed in equal ratio.

Test assay:- Required numbers of micro wells were taken and were kept in the strip holder. The strips was washed 3 times with 1X wash buffer and without letting to dry, 50 µl of dilute samples was added as per protocol of ELISA plate map. Test strips were covered with the aluminum foil and plate was kept in a humidified box at 37 0C for 1 hours. After incubation the plate was washed five times with wash buffer and dry blotted by tapping on the tissue paper. 50 µl of DEN antigen was added in each well incubated at 37 0C for 1 hours. Then plate was washed five time dry blotted. 50 µl of Anti DEN Monoclonal antibody HxB (biotin labeled) was added in each well and incubated at 37 0C for 1 hours, after incubation again washed five times, dry blotted.

Afterward 50 µl of Avidin –HRP was added into each wells and incubated at 37 0C for 30 minutes. After incubation plate was washed again. 100 µl TMB substrate was added to each well in dark and incubated at room temperature in dark for 10 minutes. Then the reaction was stop by adding 100µl stop solution and absorbance was measured at 450nm within 10 minutes.

Test validity: - The absorbance of NC should be less than 0.18 and of PC is more than 6 times the OD of NC.

Interpretation of the Result:-

- If sample OD ≤ NC OD x 2.0 = sample negative.
- If sample OD ≥ NC OD X 3.0 the sample was considered as positive.

Statistical Analysis:- Mean, standard error, standard deviation and significance (p) value were determined to find out whether the findings were statistically significant or not by using one way anova.

3. Results

Out of total number of 336 samples tested, 39 samples were found positive for Dengue NS1Ag as shown in table no.1. So, the Sero-prevalence of dengue NS1Ag cases in Chhotaudepur was 11.61%.

Table 1: Sero-diagnostic Test Results by Dengue NS1 Ag ELISA

NS1 Ag ELISA	Number of Case	Percent expression in total
Positive	39	11.61%
Negative	297	88.39%
Total	336	

In sex wise distribution higher number of dengue positive cases was observed in males (13.57%, n= 22) than in females (9.76%, n= 17) as shown in table no. 2.

Table 2: Sex-wise Distribution of Dengue Sero-positive Cases

Sex	Number of samples taken	Sero-positive cases (%)	% of sero-142 positive cases in total 143
Male	162	22 (13.57%)	6.55%
Female	174	17 (9.76%)	5.1%
Total	336	39 (11.012%)	11.61%

Age wise distribution of dengue sero-positive cases is shown in table no.3. Where the highest number of dengue cases were found to be belonged from the younger age group of 20-29 years (5.356%) and lowest was from the childhood age group of 10-19 year (1.79%). There was a significant relationship between the number of total test done to the number of total positive cases of different age group as indicated by the p- value which is 0.0134 (< 0.5).

Table 3: Age wise Distribution of Sero-positive Dengue Cases

Serial number	Age	Total number of samples	No. of Positive samples	% of Positive cases
1.	< 10 year	12	1	0.31%
2.	10-19 year	49	6	1.79%
3.	20-29 year	121	18	5.356%
4.	30-39 year	62	5	1.49%
5.	> 40 year	92	9	2.68%
Total		336	39	11.61%
Over all		336	39	11.61%
Mean (1-5)		67.2	7.8	
Standard Deviation (1-5)		41.5776	6.3797	11.61%
Std. Error (1-5)		18.5941	2.8531	11.61%
P-value		0.0134		

Month wise distribution of dengue NS1 Ag ELISA cases is shown in table no. 4 and in figure no 6. The maximum numbers of dengue cases 18 out of 105, were found in the month of October; followed by 9 out of 47 cases in month of November. The lowest numbers of dengue cases 1 out of 12 was detected in the month of June and December. There was a significant difference among the dengue cases during different months of the year (Table 4).

Table 4: Month wise Distribution of Dengue NS1 Ag Sero-positive Cases

Month	Total test	Positive	P- value
June	12	1	0.004
July	54	2	
August	51	3	
September	55	5	
October	105	18	
November	47	9	
December	12	1	
Total	336	39	

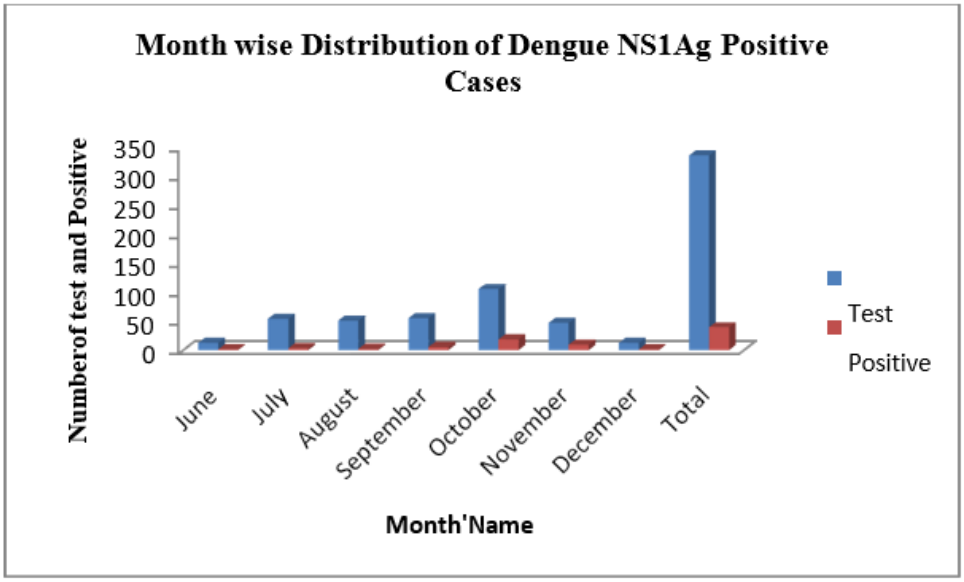

Figure 7: Month wise Distribution of Dengue NS1 Ag Cases.

Among the total 20 dengue suspected cases tested for Dengue IgM ELISA test only 3 cases were found positive for Dengue IgM ELISA as shown in table no.5, so have 12.23% sero-prevalance.

Table 5: Sero-diagnostic Test Results by Dengue IgM ELISA

IgM ELISA	Number of Case	Percent expression in total
Positive	3	12.23%
Negative	17	87.76%
Total	20	

4. Discussion

In present study, a total of 336 sample tested, 39 (11.61%) was confirmed positive for dengue NS1 antigen. Therefore sero-prevalence of Dengue was 11.61% which is in accordance with the Madkey et al., 2021 where they found the 12.37% of Dengue sero-prevalence in Gondia district of Madhya Pradesh during their study from October 2018 to September 2020. Further the study conducted by Padbidri et al, 1996 and Lata Patel et al, 2013 also found 15.4% and 16.3% dengue seroprevalence respectively [8].

Sex wise Distribution of Sero-positive DENV Cases

In sex wise distribution higher predominance of dengue cases were found in male (13.57%) than in female (9.76%). This finding is in accordance with the earlier studies conducted by Tripathi et al, 2008 and Goswami et al., 2018 where they also shown the male predominance [9, 10]. This high prevalence of dengue infection in males can probably be due to more exposure during higher involvement in outdoor activities comparison to females.

Age wise Distribution of Sero-positive DENV Cases

The higher prevalence of the dengue infection was found in age group of between 20-29 years and lowest was in the

childhood age. Similar result was also shown by Gupta et al., 2006, Kumar et al., 2010 where they revealed maximum dengue cases in under the age group of 21-30 years with male preponderance [11, 12]. The reason of this predominance of dengue infection in young male was supposed to be the involved in more of outdoor activities, hence more exposed for mosquito bites [13].

Month wise Distribution of Dengue NS1Ag Positive Cases
In month wise distribution of dengue cases the maximum numbers of dengue cases 18 out of 105 test were found in the month of October; followed by in month of November. So this study showed that the dengue infection start spreading in the month of July and August, reached at its peak in the month of October and slowly tapered toward the month of December. Thereby the seasonal transmission of dengue infection found to increase in the monsoon and post monsoon season which is in accordance with the study done by Gupta et al., 2006, Patankar et al., 2014 [11, 14]. This also indicates active viral transmission in rainy season which is the breeding period of the Aedes aegypti mosquito [15].

Only total 20 dengue suspected cases were tested for Dengue IgM ELISA from the month of June 2022 to December 2022, among 3 cases were found positive. This is mainly because most of the patients visit the Hospital in early stage of their illness.

Therefore dengue NS1Ag ELISA test is an index test for early diagnosis of patient and epidemiological purposes for earlier prevention of outbreak. Schilling et al., 2004; Ampaiwanet et al., 2008 also showed the same usefulness of the dengue NS1 antigen test. Dengue NS1 Ag ELISA test have found to have the similar detection rate of dengue virus as that for PCR [16, 17].

5. Conclusion

As the maximum no. of dengue cases was found in month of October 2022 and then tapers gradually towards the month of December, this showed that temperature and humidity condition helps mosquitoes to breed which occur due to presence of stagnant water after. Therefore the Government have to take the proper, effective preventive and control measures prior to the beginning of monsoon to reduce the occurrence of dengue in the community. The most of the dengue cases were found to be belonged from the younger age. Further only total 20 suspected serum samples was tested for IgM ELISA among only 3 was positive. This showed the immense need of an early and rapid test for diagnosis of dengue infection. Where the NS1 antigen ELISA test appears as best choice in term of its rapidity, ease of performance, higher sensitivity, and cost effectiveness for early detection of dengue infection

References

1. Tchuandom, S. B., Tchadji, J. C., Tchouangueu, T. F., Biloa, M. Z., Atabonkeng, E. P., et al. (2019). A cross-sectional study of acute dengue infection in paediatric clinics in Cameroon. BMC public health, 19, 1-7.
2. Gubler, D. J. (1998). The global pandemic of dengue/dengue haemorrhagic fever: current status and prospects for the future. Annals of the Academy of Medicine, Singapore, 27(2), 227-234.
3. Peeling, R. W., Artsob, H., Pelegrino, J. L., Buchy, P., Cardosa, M. J., et al. & Yoksan, S. (2010). Evaluation of diagnostic tests: dengue. Nature Reviews Microbiology, 8(Suppl 12), S30-S37.
4. Datta, S., & Wattal, C. (2010). Dengue NS1 antigen detection: A useful tool in early diagnosis of dengue virus infection. Indian journal of medical microbiology, 28(2), 107-110.
5. Bhatt, S., Gething, P. W., Brady, O. J., Messina, J. P., Farlow, A. W., et al. (2013). The global distribution and burden of dengue. Nature, 496(7446), 504-507.
6. Naik, N. S., Murarka, S. V., Sanghrajka, D., Shah, B. S., Raval, R. J., et al. (2021). Dengue infection profile in Gujarat, West India: A recent report. Journal of Applied Biology and Biotechnology, 9(5), 96-100.
7. Mistry, M., Goswami, Y., Chudasama, R. K., & Thakkar, D. (2015). Epidemiological and demographic characteristics of dengue disease at a tertiary care centre in Saurashtra region during the year 2013. Journal of vector borne diseases, 52(4), 299.
8. Padbidri, V. S., Mahadev, P. V. M., Thakare, J. P., Pant, U., Ilkal, M. A., et al. (1996). Virological and entomological investigations of an outbreak of dengue fever in Dhule district, Maharashtra. INDIAN JOURNAL OF MEDICAL MICROBIOLOGY, 14, 25-32.
9. Tripathi, P., Kumar, R., Tripathi, S., Tambe, J. J., & Venkatesh, V. (2008). Descriptive epidemiology of dengue transmission in Uttar Pradesh. Indian pediatrics, 45(4), 315.
10. Goswami, L., Chowdhury, R., & Rasul, E. S. (2018). Seroprevalence of dengue infection in a tertiary care hospital in Assam.
11. Gupta, Ekta, Lalit Dar, Geetanjali Kapoor, and Shobha Broor. "The changing epidemiology of dengue in Delhi, India." Virology journal 3 (2006): 1-5.
12. Kumar, A., Rao, C. R., Pandit, V., Shetty, S., Bammigatti, C., et al. (2010). Clinical manifestations and trend of dengue cases admitted in a tertiary care hospital, Udupi district, Karnataka. Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine, 35(3), 386.
13. Lall, H., Gupta, P., Debbarma, M., Sharma, P., Ansari, S. K., et al. (2016). Sero-prevalence of dengue in tertiary care hospital in Delhi. Int J Curr Microbiol Appl Sci, 5(6), 439-445.
14. Patankar, M., Patel, B., Gandhi, V., Shah, P., & Vegad, M. (2014). Seroprevalence of Dengue in Gujarat, Western India: A study at a tertiary care hospital.
15. Patel, L. R. (2013). Sero prevalence of Dengue NS-1 Antigen in Tertiary care hospital, Ahmadabad. Indian Journal of Basic & Applied Medical Research, 7(2), 694-701.
16. Gubler, D. J. (1998). The global pandemic of dengue/dengue haemorrhagic fever: current status and prospects

for the future. Annals of the Academy of Medicine, Singapore, 27(2), 227-234.

17. Chuansumrit, A., Chaiyaratana, W., Pongthanapisith, V., Tangnararatchakit, K., Lertwongrath, S., et al. (2008). The use of dengue nonstructural protein 1 antigen for the early diagnosis during the febrile stage in patients with dengue infection. The Pediatric infectious disease journal, 27(1), 43-48.