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Dengue Viral Augmentation among the Population of Few Districts of Assam and Neighbourhood Visiting Tezpur Medical College & Hospital

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ABSTRACT

Dengue virus (DENV) has rapidly expanded its range through tropical and subtropical regions in recent years. This pathogen causes acute febrile illness (dengue fever, DF) and severe bleeding disease (dengue hemorrhagic fever, DHF) in humans. In this study we are trying to analyze the data obtained from the diagnosis conducted upon the Dengue suspected patients visiting tertiary care hospital, Sonitpur, Assam and to give a scientific evaluation to the pattern or spread of this epidemic disease. A total of 361 serum samples are analysed from suspected dengue cases during this study period. This study basically gives our research team to go more investigating the dengue viral expansion in coming days as we were able to discriminate among the patients on the basis of primary and secondary infections, what they were previously not examined for. Significant clue for the presence of secondary dengue viral infections (17.3%) among 10 different districts is found.

Keywords: Dengue, Viral Research and Diagnostic Laboratory (VRDL), Tezpur Medical College & Hospital (TMCH)

INTRODUCTION

"*It's in the misery of some unnamed slum that the next killer virus will emerge*" by noticing an worthy quote by former US President Barack Obama, one can call it's a day when the urban commons of the world has understood that any disaster could be unleashed by a tiny virus reincarnating as an opportunistic giant. But, such giant could be a more than a disaster for the people of country like India whose residents live in sub-urban or rural area as percentage of 66.46 of total population, who are struggling yet to get basic medical facilities and health hygiene till this moment. Due to such health-compromising situations only, the residents of this developing country repeatedly face the wrath of viral diseases like Dengue, previously in certain months, now almost for the whole year.

Dengue virus (DENV) has rapidly expanded its range through tropical and subtropical regions in recent years. This pathogen causes acute febrile illness (dengue fever, DF) and severe bleeding disease (dengue hemorrhagic fever, DHF) in humans. DF and DHF, with an estimated annual infection rate of 100 million and

approximately 500,000 deaths, are the most important health issues in tropical and subtropical regions. Dengue virus is transmitted between humans and the arthropod host (mosquito), which shows that humans are not the terminal host [1]. The virus grows in the mosquito gut and migrates to the salivary glands. When an infected mosquito feeds on a healthy person, the virus is inoculated subcutaneously [2,3]. Dengue virus primarily propagates in skin dendritic cells, and subsequently virus proliferation is thought to occur in target cells such as those of the monocyte/macrophage lineage [4].

The establishment of infection requires the entry of DEN virus into cells, followed by release of nucleocapsid. This is achieved by the fusion of viral membrane with a cellular membrane [5]. In most cases of initial infection, the host develops dengue fever, which has mild prognosis. Cross-neutralizing antibodies against different serotypes disappear within a short period, and a virus of another serotype may cause re-infection (secondary infection). When secondary infection by different serotypes occurs, the immune complexes of the viruses with cross-reactive antibodies produced during primary infection enhance viral infection mediated through Fcγ receptor dependent incorporation of the virus into host cells which may lead to DHF or dengue shock syndrome [6–11]. Monocyte macrophages are the principal target cells for dengue viruses.

Practically, the viral infections could be detected in terms of interaction of NS antigen to IgM or IgG antibodies present in human blood cells. As an alternative, the detection of viral antigens has been proposed, and more recently attention has been focused on nonstructural protein 1 (NS1) of DENV [12, 13]. This protein has been identified as a highly conserved glycoprotein expressed in either membrane-associated or secreted forms. It possesses not only group-specific but also type-specific determinants and has been recognized as an important immunogen in dengue viral infections. On the other hand IgM antibodies are detected as the first immunoglobulin isotype to appear in. These antibodies are detectable in 50% of patients by days 3-5 after onset of illness, and may increase up to 80% by day 5 and 99% by day 10 reported in some cases. IgM levels peak about two weeks after the onset of symptoms and then decline generally to undetectable levels over 2–3 months. Anti-dengue serum IgG is generally detectable at low titres at the end of the first week of illness, increasing slowly thereafter, with serum IgG still detectable after several months, and probably even for life [14-16].

In this study we are trying analyze the data obtained from the diagnosis conducted upon the Dengue suspected patients visiting Tezpur Medical College & Hospital (TMCH), Bihaguri, Sonitpur, Assam from its nearby area and to give a scientific evaluation to the pattern or spread of this epidemic disease. Here, some studies had been performed to discriminate primary and secondary DENV infection using the ratio of IgG and IgM at the various days of symptoms onset [17-21]

MATERIALS AND METHOD

Population and case definition

A retrospective study was performed at the Viral Research and Diagnostic Laboratory (VRDL)at Department of Microbiology, Tezpur Medical College and Hospital for the period 2018-2019 (September 18 to October 19). A total of 361 serum samples from suspected dengue cases during this period attending OPD or admitted in the various wards or ICUs of Tezpur Medical College and Hospital were tested for the confirmation of the disease Dengue. The case ranges from juveniles ageing 0 to adults till 100 years old. A suspected case of dengue was considered a patient with acute febrile illness and signs and symptoms suggestive of Dengue; headache, retro-orbital pain, myalgia, arthralgia, rash and haemorrhagic manifestation, etc [22] following the Case Report Forms provided by National Institute

of Epidemiology, Chennai (NIE). Serum samples from these patients were tested for either Dengue NS1antigen using dengue NS1 antigen capture ELISA(ERBA-ELISA) and dengue IgM antibody by dengue IgM capture ELISA (MAC-ELISA) (National Institute of Virology, Pune, India) on the basis of viremia as well as other conditions. ELISA tests were performed as per the manufacturer's instructions. Consent of the Institutional Ethical Committee was taken for the study.

A primary infection was defined as either NS1Ag (positive/negative) followed by IgM (positive/negative) but a negative IgG. Secondary infection was defined as a positive/negative IgM and positive IgG [23]. As there might be provision for equivocal results of IgM ELISA, in our case detection of any of the sero components such as NS1Ag or IgM was taken positive for further serodiagnosis of probable secondary infection with IgG capture ELISA (STANDARD E IgG CAPTURE ELISA KIT, SD Biosensor). IgG ELISA was performed on those cases having dengue fever symptoms more than 10 days for discriminating secondary infection [16]. Blood samples (2-5ml) preferably, were collected from each of these cases, one as soon after admission as possible and the second sample one week later or at the time of discharge or on revisit of the patient. Serum was separated and stored at -80^{0}C till the tests were performed.

DENV IgM/IgG ratio

The DV IgG/IgM ratio was calculated for sera positive for IgG and IgM by dividing the IgG index value with the IgM index value.

Study parameters

The parameters taken for this study were age groups, sex, month of the year, districts of belonging, meteorological conditions of study period for differentiation of affected people and data. The Meterological data was collected from Gramin Krishi Mausam Sewa (GKMS), Sonitpur AMFU, B.N. College of Agriculture AAU, Sonitpur, 784176 Assam.

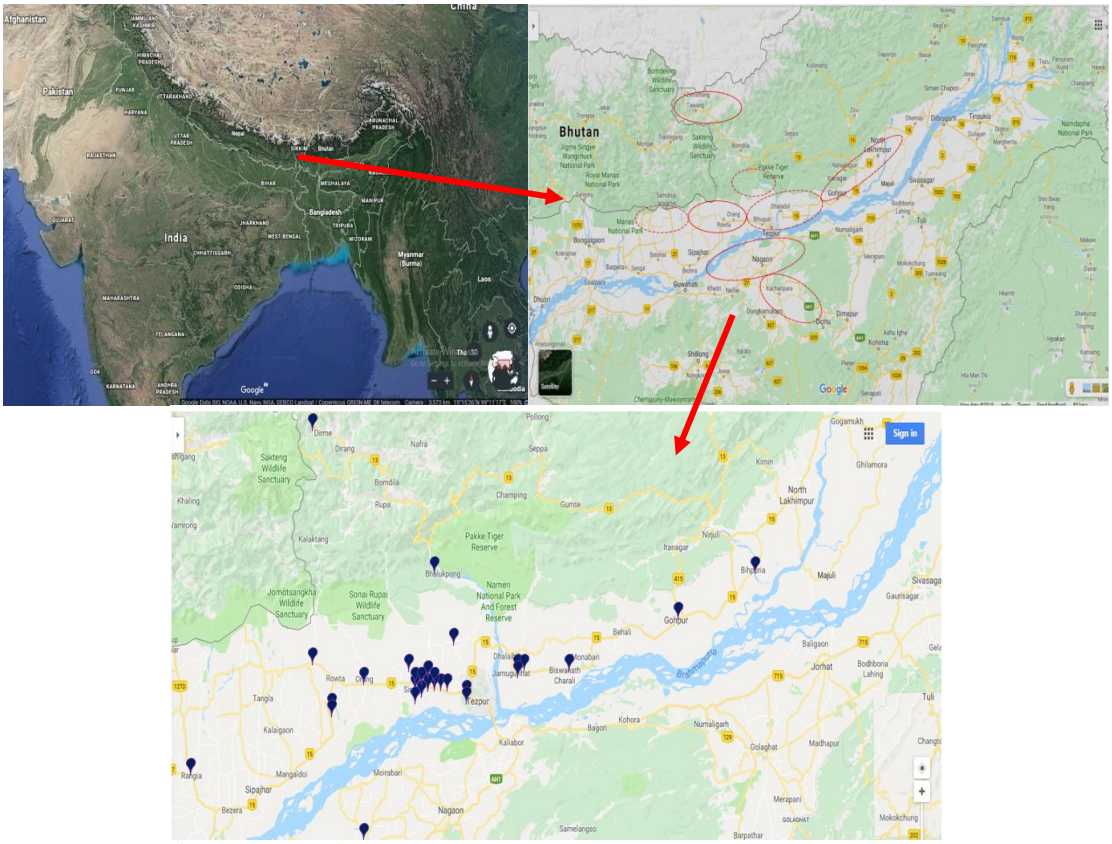
Statistics

The statistics and data analysis was done by using Microsoft Office Excel tools and Venn diagram was created online through www.meta-chart.com. Maps were cited with the help of Google Earth. Chi-square tests were used for categorical variables. Correlation and Regression analysis were also performed in necessary cases. Sensitivity, specificity, positive likelihood ratio, and cut-off point for IgG/IgM ratio were determined using Receiver Operator Characteristic (ROC) curve. Area under curve (AUC) was calculated for each ROC curve to find the suitable curve to get a proper cut-off point.

RESULTS

It was observed from the initial study upon the patients regarding their residence that most of the populations were habitants of sub-urban or rural area (98%). The suspected people visiting TMCH came from many of the districts of northern and southern bank of Brahmaputra river as well as nearby states. The districts of Assam were namely, Sonitpur, Biswanath, Udalguri, Baksa, Lakhimpur, Darrang, Karbi Anglong, Nagaon and Morigaon. From Arunachal Pradesh cases were to be habitants of Tipi and Bhalukpong from West Kameng district and even from Tawang district. They constituted the percentage of whole population as 77.56, 8.31, 4.7, 0.27, 3.6, 0.27, 1.38, 2.77, 0.27 and 0.83 %.

Among the population of 361, when the population was observed within age groups of (0-10, 11-20, 21-40, 41-60, 61-80 and 81-100) it was observed that they belong in percentages of the total number i.e., 1.93, 18.00, 55.95, 18.55, 4.98 and 0.55% (Fig.2).



Picture 1.Distribution of cases suspected for Dengue virus among the patients visiting TMC & H during 2018-19 (Positive cases:43)

Fig.1. Distribution of suspected persons visiting TMCH according to their residences.

Fig. 2 Pie diagram showing the variety of ages of population tested for Dengue ELISA tests.

Again, within age groups of (0-10, 11-20, 21-40, 41-60, 61-80 and 81-100) was studied according to the genders, the male patients felt in those groups as 0.83, 9.14, 28.53, 7.47, 2.493 and 0.55% respectively. The female patients felt in those groups were as 1.10, 9.41, 27.70, 10.24 and 0% respectively (Fig 3). The chi-test and chi-square test for this study was found to be 0.6037 and 3.6302, which gave a *p*-value of 0.162 showing a less significance of the age to disease progression among whole population.

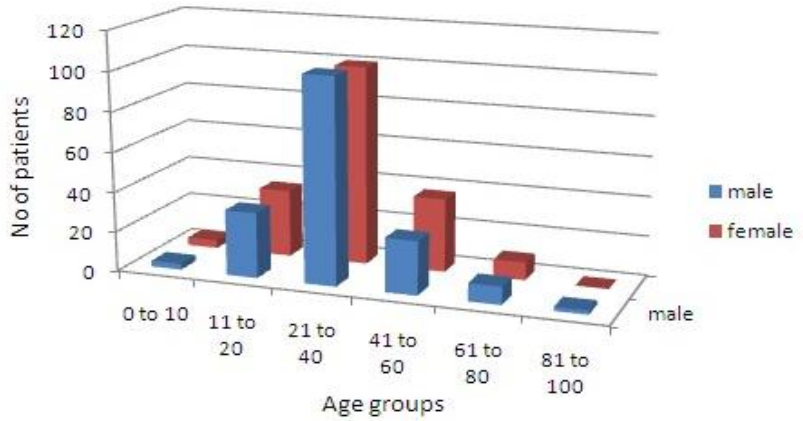


Fig.3 Figure showing the number of population tested for Dengue ELISA tests varied by male and female.

When the population was tested by NS1 antigen detection and IgM capture ELISA, 11.9% population came out as Dengue positive, 68.42% as negative and 19.66% patients were found to equivocal to MAC-ELISA dengue tests. However, only 2 patients were detected as NS1 positive throughout this study period.

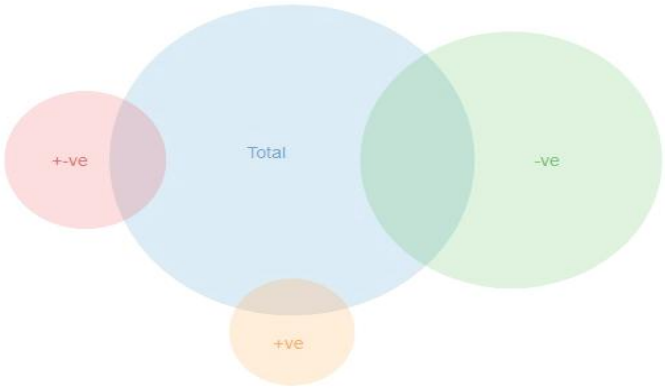


Fig. 4:The Venn diagram showing the distribution of results of the tested samples. The term "Total", "+ve", "-ve" and "+-ve" represents the total no of population, Dengue positive person no, Dengue negative no and equivocal case nos respectively.

When the positive results were observed with the progression on months starting from September, 2018 it was known that the number of patients gradually went high till August, 2019 and a sudden fall occurred after September, 2019.

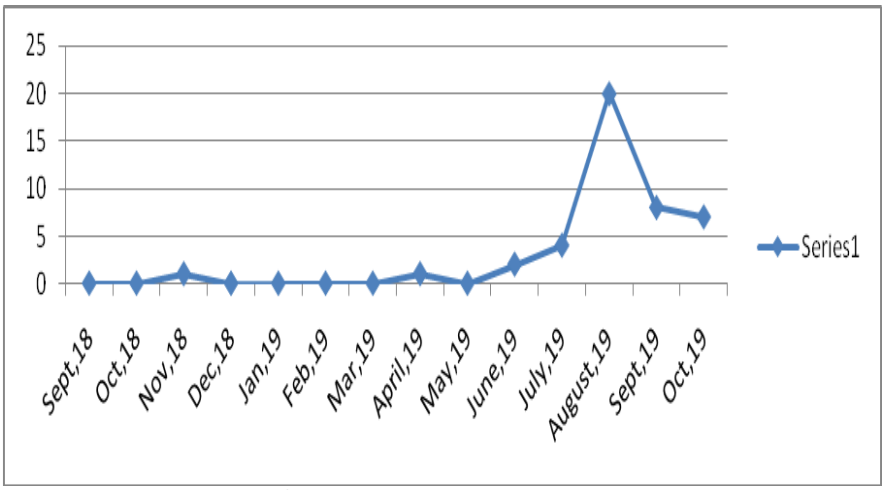


Fig. 5 Distribution of Dengue positive patients throughout the year 2018-19.

Out of the 361 subjects though, 173 of them had suffered multiple symptoms of acute febrile illness related to dengue infection, but all total of 43 patients were defined as positive of dengue infection by IgM or NS1 Antigen based ELISA. Within the positive cases 65.11% were male and 34.88% were female patients.

When total positive cases were observed with age groups it was found that they fall under groups as 0, 16.27, 62.79, 18.60, 2.32 and 0% in (0-10, 11-20, 21-40, 41-60, 61-80 and 81-100). A polynomial regression analysis showed a R^2=0.524 showing a less affect of progression of age groups on the positivity of samples as have *p*-value of 0.279 (Fig.6). Again, differentiating among male and female the groups attained male population of 0.0, 9.3, 41.86, 13.95, 0.0, 0.0 % and female as 0.0, 6.97, 20.93, 4.65 and 2.32% with age group (0-10, 11-20, 21-40, 41-60, 61-80 and 81-100). The chi-test and chi-square test for this study was found to be 0.4871 and 2.4352, which gave a *p*-value of 0.295 showing a less significance of the positive cases according to genders with various age groups.

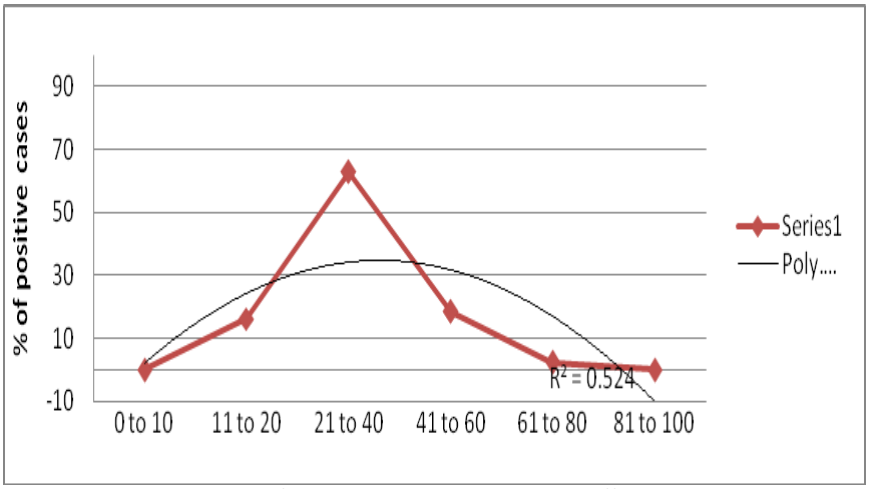


Fig. 6 Distribution of Dengue positive patients among different age groups.

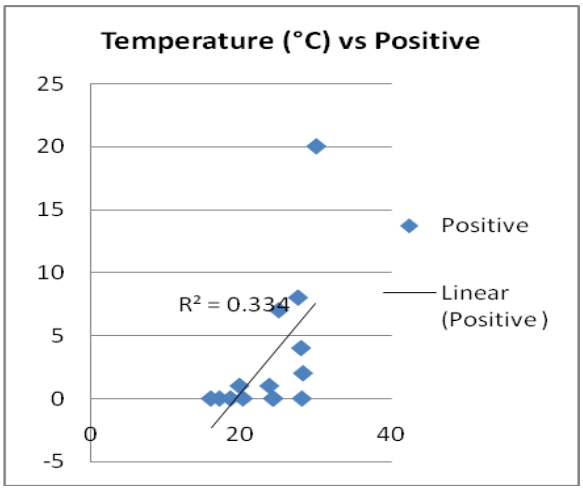
During the progression of the months of year 2018-19 the male and female positive cases were found as 0, 0, 2.32, 0, 0, 0, 0, 0, 0, 2.32, 2.32, 25.58, 13.95 and 11.62 % (male) and 0, 0, 0, 0, 0, 0, 0, 2.32, 0, 2.32, 6.97, 20.93, 4.65 and 4.65% (female) among the whole population tested for dengue viral symptoms. The chi-test and chi-square test for this study was found to be 0.4824, and 5.4917, which gave a *p*-value of 0.0641 showing minimal significance of the data per month.

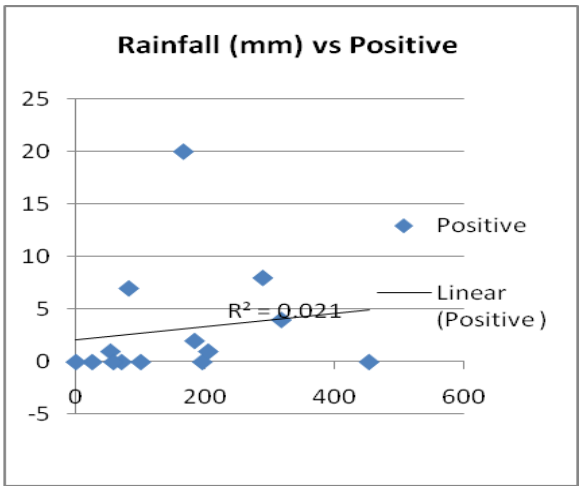
To understand external influence on expansion of dengue viral syndrome among patients' meteorological data was collected for average weather temperature, rainfall and relative humidity.

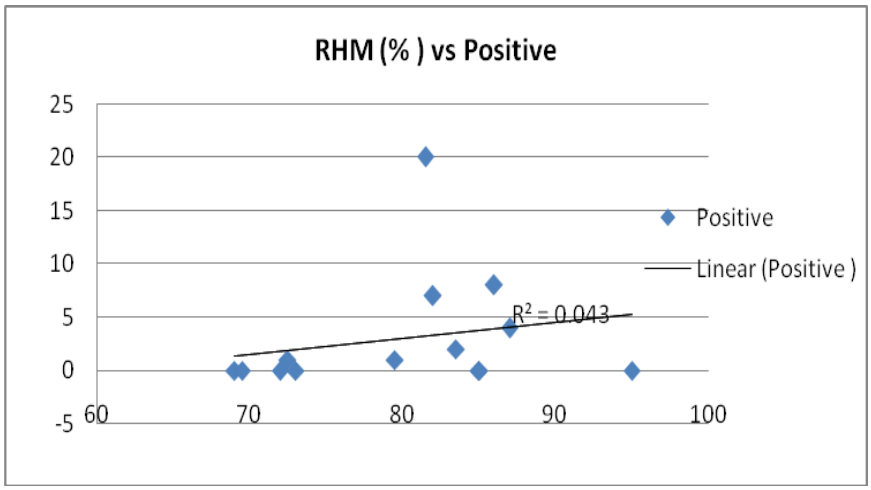
Table 1. Variation of average weather, temperature, rainfall and relative humidity (RHM) in Sonitpur district and neighbourhood.

Months	AvgTemp (°C)	Avg Rainfall (mm)	AvgRHM (%)	Positive cases(%)
Sept,18	28.05	195.6	85	0
Oct,18	24.25	70.6	95	0
Nov,18	19.8	53.5	72.5	2.32
Dec,18	17.15	25.3	73	0
Jan,19	16	0	69	0
Feb,19	18.6	58	72	0
Mar,19	20.25	100.4	69.5	0
April,19	23.75	204.8	79.5	2.32
May,19	24.25	453.6	85	0
June,19	28.2	183.6	83.5	4.65
July,19	27.95	317.9	87	9.30
August,19	29.95	166.4	81.5	46.51
Sept,19	27.55	289.2	86	18.60
Oct,19	25	81.8	82	16.27

When linear regression analysis was studied among positive cases to meteorological conditions like Temperature, Rainfall and RHM, the R-squared values were found as 0.334, 0.021 and 0.043 respectively. The *p*-values for those analyses were found to be 0.03, 0.4 and 0.5 respectively. It shows that temperature vs positive cases result having moderate level of significance. The correlation coefficients for them are as 0.578, 0.145 and 0.209 respectively which also supported the same.







When the IgM positive / equivocal / negative patients (total patient 104) under the criterion below illness more than 10 days were subjected to IgG ELISA, 17.30% of the population was founded to be suspected of secondary dengue infection. Upon studying different cut-off values to discriminate between primary and secondary infections, a value of IgG/IgM ratio 1.3 was found to be most suitable.

Table 2.This table shows the sensitivity, specificity, area under curve (AUC), LR+ calculated for different ratios of IgG/IgM for the diagnosis of secondary dengue fever. Abbreviations: LR+ positive likelihood ratio, [a]Most useful value to diagnose secondary dengue. 1.3 was chosen as the most accurate ratio of IgG/IgM to diagnose based on all the variables used here.

SlNOs	IgG/IgM Ratios	Sensitivity (%)	Specificity (%)	AUC	LR+
1	0.9	95.65	93.9	0.9903382	15.49
2	1.1	95	97.62	0.9922619	39.9
3	1.3[a]	94.44	98.84	0.998385	40.61
4	1.4	94.44	98.84	0.9922481	40.61
5	2	69.23	99	0.9763314	63
6	2.4	63.63	99	0.9472141	59.18

The study found that IgG/IgM ratio of ≥1.3 confirmed secondary infection with sensitivity of 94.44 %, specificity 98.4 % and likelihood ratio of (LR+) 40.61 as

shown in Table 2. The cut-off point had good performance because area under ROC curve was 0.998 (Fig 7).

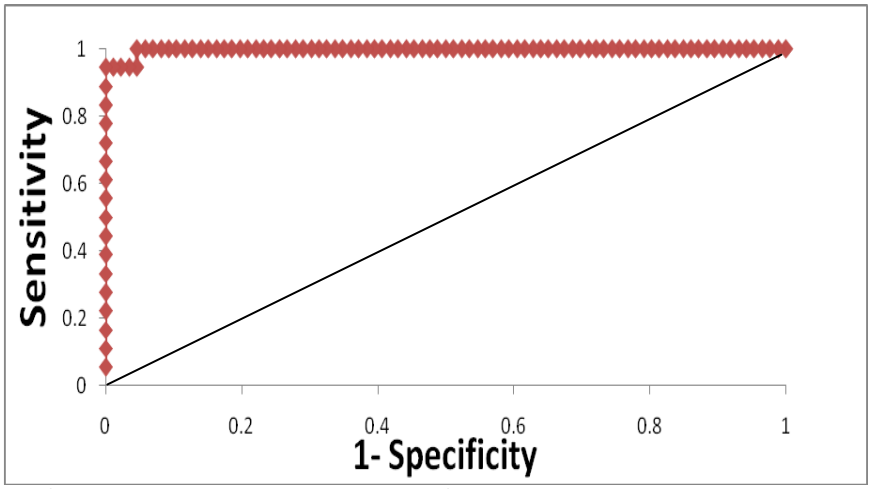


Fig 7. The figure shows the best suggested ROC curve for IgG/IgM ratio 1.3. The area under curve is 0.998.

DISCUSSION

Dengue viral expansion recently becomes capable of threatening half of the world's population and is an important public health problem in many tropical regions of the world. [24] It is observed that during the last three decades, the demographic and clinical features of dengue infections have changed drastically. [25][Assam dengue]. [Dengue fever, once known as a childhood disease and an important origin of paediatric hospitalisation in Southeast Asia [26], now stretches its palm over almost all age groups of Indian population [27]. In an outbreak in Delhi during 2003, dengue positives in the adult group outnumbered those of children although the difference in the number of positive cases was not significant compared to pediatric age group [28]. In another outbreak in Malaysia during 2006-2007, it was observed that the majority of the cases were adults in the 21to 25 years and >35 years old age groups. With this changing demography, it is possible that there are features of severe dengue leading to death that could be different from those seen in children earlier days [29]. Supporting this trend, in the present study, it was observed that the maximum cases were in the young adult age group (21-40 year) viz, 62.79% out of all positive cases or 56.23 % of the whole population visiting TMCH.

There have been reports of dengue outbreaks in most of the parts of India except, the extreme northern and north-eastern States, which could be due to their unique temporal and spatial characteristics. But, already north-eastern states such as Assam or Manipur had experienced severe hazard due to Dengue outbreak in recent past and starts to suffer every year till date, to be said as a victim of climate change. The transmission of mosquito borne diseases is climate sensitive as the mosquitoes need water to breed and ambient temperature is critical to the larval development and the feeding behavior of adults. As per the theoretical models, the transmission patterns of dengue are influenced by temperature and precipitation. Temperature affects the egg viability, larval development, adult longevity and dispersal, whereas rainfall affects the abundance and productivity of the breeding habitats of Aedes mosquitoes [30]. According to the State Action Plan for Climate Change [31], the annual mean temperature in the state has increased by 0.59 degrees Celsius over the last 60 years (1951 to 2010), and is likely to increase by 1.7-2.2 degree Celsius by 2050. Our result directly reflects the influence of temperature increase in augmentation of dengue fever

cases by showing highest value of 46.51% infection out of all positive cases in the month of August 2019, which was the warmest month of that year. (Though not identified as highly significant from present *p*-values of 0.4 and 0.5 of monthly average rainfall and relative humidity data respectively with all over positive cases, the present study however becomes supportive to other case studies related to these meteorological parameters. The Dengue positive case rate was higher as 46.51 and 18.6% during the months of September, 2019. As expected the average rainfall and relative humidity was seen to be 289 mm and 86%. In a detailed study conducted in Nepal, researchers found that overall; the factor temperature-rainfall effect had a more significant influence on vector indices compared to relative humidity. Again, the regression models showed that relative humidity has a greater impact on meteorological good [32]. Whereas, in January or February, 2019 positive rate was nil and average rainfall and relative humidity was observed as 0mm and 69%, 58 mm and 72% which clear a pattern of their influences in DENF progression.

In the northeast region (NER) of India, serological survey conducted during 1963 revealed Dengue activity in the Lohit district of Arunachal Pradesh (AP) and Darrang district of Assam [33, 34]. Subsequently, another report of Dengue type-2 in Assam and Nagaland appeared during the nineties [34–36] and till 2011 presence of dengue cases became evident in other NER states too. Though DENV-2 was predominantly a major serotype in these area, Khan et al 2014 reported presence of DENV-3 and even co infection by DENV1 and 3 in an outbreak near Pachighat, an Assam-AP border area [37]. So, secondary dengue viral infection has become an enthusiastic topic in these regions presently. The TMCH VRDL being established at foothill of Himalaya as well as northern valley of river Brahmaputra, was able to get a diverse population of suspected cases from 9 different districts of Assam and 1 from AP too. The declaration of 17.3 % out of 104 nos of initially discriminated patients as probable secondary infection might be an important outcome of this piece of research work. Other studies have also reported a higher number of secondary dengue cases. Such as, Changal et al. [23], conducting an observation of acute primary and secondary infection in Northern India reported 66% presence of secondary infection in their cases. In present study acute primary cases were confirmed for 11.9% whereas 19.66% were found to be equivocal. Out of them some came to visit the hospital when already 10 days of dengue viral symptoms was carried. So, selecting final 104 samples include patients visiting after 10 days of syndrome plus all IgM negative/equivocal cases. Higher number of secondary dengue infections occurs only in Dengue endemic countries. Pathogenesis in dengue is linked to the host immune response, which is triggered by infection with the virus. Primary infection is usually benign. Secondary infection with a different serotype or multiple infections with different serotypes may, however, cause severe infection [38]. This leads secondary dengue to be clinically more apparent and thus more people seeking medical care. The results from different studies reiterates the well accepted dictum that IgM ELISA is the test of choice after first 5 days of Illness [39] and this helped to define a primary infection. Hence, samples selected from 10^{th} day of illness become a good criteria for getting secondary cases. But, to identify a secondary DENV infection IgG/IgM ratio is more crucial and practiced every research laboratory. IgG/ IgM ratio has been studied in many studies for early differentiation of primary and secondary dengue. Ratios done at different time stages of the disease have revealed different proposed cut offs [17,18, 19]. In present case, we found 1.3 or higher as the best cut of point and the respective ROC curve gave the highest AUC. A study in Bali, Indonesia found IgG/IgM ratio of ≥ 1.1on the day 5–7 of disease is a good predictor of secondary dengue infection.

Again, Study in Malaysia found that the best cut off point for secondary infection was ≥ 2.0 [40]. But from an elaborated study among good population (114) a team from Indonesia found a best cut of ≥ 1.14 [41]. Different ratios found in these studies might be due to different settings and seroepidemiologics [17, 18, 42].

CONCLUSION

This study basically gives our research team to go more investigating the dengue viral expansion in coming days as we were able to discriminate among the patients on the basis of primary and secondary infections, what they were previously not examined for. We got significant clue for the presence of secondary dengue viral infections (17.3%) among 10 different districts. Though this study could be a benchmark for practicing other molecular biological approach to go inside this population, but this study was limited as the proper date of onset of viremia was unable to achieve as the study population were belonging to very rural parts of northern Assam. Moreover, as now-a-days viral fever is widespread among every class of people, patient often unable to mention the symptoms similar to dengue fever. Furthermore, RT-PCR and pathological approach is planned to carry out this study by our team.

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