“Prevalence of Dengue And Chikungunya Coinfection In Kalaburagi Region” Karnataka India

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Abstract:

Dengue and Chikungunya are the most common Arboviral infections and have caused several fatal outbreaks in the Gulbarga region. This study aims to explore the prevalence of Dengue and Chikungunya co-infection among Gulbarga population since it presents very similar signs and symptoms in the early stages of acute febrile illness. Blood samples were collected from patients presenting symptoms of acute febrile illness during monsoon season i.e., from Oct and Nov-2019. Sample were tested for Dengue and Chikungunya IgM ELISA using standardized kits from NIV-Pune. Positive cases were then confirmed by the Dept. of Neuro-Microbiology NIMHANS-Bangalore. About 519 samples were included in the study, of which 234(45.1%) were male and 285(54.9%) were female patients. Out of 519 samples, 136 samples were positive for Chikungunya and 58 samples positive for Dengue and 22 were positive for both Dengue and Chikungunya, concluding a co-infection. Patients of age group between 31-40 and 21-30 years showed highest positive case of Chikungunya (33%). Taluka wise distribution of positive cases shows maximum positivity from Gulbarg Urban region with 55% and 27.2% Dengue and Chikungunya respectively. Our study suggest the prevalence of co-infection (Dengue and Chikungunya) rather than mono infection of either Dengue or Chikungunya, which requires laboratory confirmation of dual infection followed by strict treatment strategy and preventive measures of regular house hold monitoring of water storage and emptying if any breeding of mosquitoes exists.

Key words: Dengue, Chikungunya, Dengue& Chikungunya co- infection, IgM ELISA, Seroprevalence

1.INTRODUCTION:

The viruses spread by Arthropod vectors are termed as Arboviruses (Arthropod-borne virus) most common vectors are mosquitoes, ticks, and sand flies (1). Flaviviridae, Togaviridae, Bunyaviridae, and Reoviridae are the families of viruses included in the arbovirus group. Common feature are shared among these large families of viruses such as, rapid adaptation of these viruses to ever-changing host and environmental conditions is promoted by an RNA genome. Thus, West Nile virus, Dengue virus, and Chikungunya viruses are largely responsible for the recent growth in geographic range of emerging viral infections. The epidemiology, presentation, and diagnosis of arbovirus infections and therapeutic approaches to treat them are reviewed by many studies (2). Hundreds of viruses, designated as arboviruses, are transmitted by arthropod vectors in complex transmission cycles between the virus, vertebrate host, and the vector. The interactions between the biological and environmental factors that play a vital role in pathogenesis, disease outcomes and Arboviral transmission. Mosquito-borne arboviruses are focused in the review and current knowledge of the factors and underlying mechanisms are discussed that influences pathogenesis and transmission of arboviruses and pathway and critical factors that can potentially become targets for intervention and therapeutics(3).

1.1 CLINICAL PRESENTATION OF DENGUE:

In India first confirmed outbreak of dengue occurred in Kolkata 1963-64. The clinical manifestations of dengue infections range from asymptomatic to severe illness that may be fatal if not treated properly. The symptomatic cases are categorized as Undifferentiated Febrile illness (UF), Dengue Fever (DF), Dengue Haemorrhagic Fever (DHF), Dengue Shock Syndrome (DSS) and Unusual Dengue (UD) or Expanded Dengue Syndrome (EDS) (4). Clinically UF cannot be diagnosed but it is evident based on serology or virology. DF is considered to be a mild disease associated with massive bleeding and death is rarely reported. DHF – It manifests clinically similar to DF during the febrile phase. Elevation in vascular permeability (plasma leakage) is the distinctive feature that differentiates DHF from DF. Ascites and pleural effusion is caused by the selective plasma leakage into the pleural and peritoneal cavities. DSS – presentations are the same as those in DHF but the plasma leakage is so severe that the patient develops shock. (4). It is a dangerous complication of dengue infection and is associated with high mortality. Severe dengue occurs as a result of secondary infection with a different virus serotype. Increased vascular permeability, together with myocardial dysfunction and dehydration, contribute to the development of shock, with resultant multi organ failure. The onset of shock in dengue can be dramatic and its progression could be relentless. The pathogenesis of shock in dengue is complex. It is known that endothelial dysfunction induced by cytokines and chemical mediators occurs (5). UD – most of the unusual cases are DHF cases with prolonged shock or DHF inpatients with co-morbidities or DHF together with other infections. DHF/DSS accounts for about 10% of the symptomatic cases [5].

The standard tourniquet test, the Winthrobe technique, involves raising the blood pressure to midway between systolic and diastolic pressure for five minutes. Then release pressure and wait about one minute or until the circulation comes back to normal. Read the result. The positive test is ≥ 10 petechiae/mm². The Daisy technique for tourniquet test is easier and can be used in children > five years old and adults. In this technique, pressure is applied to 80 mmHg for five minutes and then released and the result read as in the Winthrobe technique [6].
Chikungunya Clinical Manifestations:

Chikungunya fever affects people of all age groups, and both genders equally. The incubation period ranges from 3 to 12 days. Prodromal symptoms are rare. Abrupt onset is usually seen in acute stage with high-grade fever (usually 102-105 °F), severe arthralgia’s, myalgia’s, and skin rash, headache, throat discomfort, abdominal pain, and constipation may also be evident. Conjunctival suffusion, persistent conjunctivitis, cervical, or sometimes generalized lymphadenopathy may be present.

Chikungunya virus belongs to the same family of viruses (Togaviridae) as Rubella virus, for which some of these complications have been described. Uterine contractions or fetal heart rate abnormalities is caused by chikungunya infection with characteristic high fever which might promote spontaneous or induced preterm delivery (cesarean for fetal salvage). Third-stage haemorrhaging or vaginal bleeding during pregnancy is the clinical manifestation of the haemorrhagic syndrome described at the onset of infection, as reported for Dengue virus (7). Laboratory diagnosis of Dengue virus is similar to other arbovirus, NS1 antigen detection from 1-18 days, in primary infection antibody titre is low, IgM appears first after 5 days of fever and disappear within 90 days. IgG is detectable at low titre in 14-21 days of illness and then it slowly increases. IgG titre antibody titre rises rapidly in secondary infection, low levels of IgG remains detectable for over 60 years. NIV-Pune MAC ELISA kit has sensitivity and specificity of 90% and 98% respectively. Haemagglutination inhibition test, complement fixation test and neutralization tests are other antibody detection assays(8). Chikungunya Virus is a single stranded RNA virus 60-70nm in diameter with a phospholipid envelope with 3-7 days of incubation period affecting all age groups. With, a characteristic high grade fever often reaching 102°F-105°F. The fever may return for 1-2 days after 4-10days hence called (Saddle back fever). Incubation period is about 5 days (3-7 days). Most common symptoms are fever and severe joint pain due to arthritis which is a polyarticular, migratory and oedematous joint swelling predominantly affecting the small joints of wrists and ankles, other symptoms include headache, muscle pain, tenosynovitis or skin rashes, older adults (≥65 years) new borns and persons with underlying hypertension, diabetes or heart disease are high risk groups(9). Chikungunya virus disappeared from the country after last reports from Maharashtra 1973(10). Chikungunya can spread with ease and causes a high percentage of clinical cases with a very high attack rate in an immunologically poor population. However, chikungunya is overshadowed by dengue in outbreak situations and in dengue endemic areas. Diagnosis of chikungunya is equally important, as it would help to estimate the disease burden and, in turn help with designing intervening strategies as co-circulation and coinfections of these two viruses are reported. (2) Chikungunya exists as a single serotype thought to confer life-long immunity in recovered individuals. However, sufficient variation to discern three genotypes, namely the Enzootic West African (WAf), and east/central/south African (ECSA), and the epidemic Asian genotypes. The ECSA recently gave rise to the Indian Ocean Lineage (IOL) responsible for epidemics in the Indian Ocean Island, mainland India and Europe beginning in 2004. (11) There is a mutation in viral genome which is responsible for re-emergence of chikungunya disease in few recent years. New mutational virus contains replacement of valine by alanine in 226 position of E1 glycoprotein gene (8). Laboratory diagnosis of chikungunya is similar to other arboviruses, viral isolation (in mosquito cell line), RTPCR are best for early diagnosis (0-7 days), serum antibody detection of IgM or a fourfold rise in IgG titre is significant, IgM appears after 4 days and lasts for 3 months, IgG appears late after 2 weeks and persists for years.

II.1 Material Method:

Samples were referred to the public health laboratory for diagnosis of disease hence, 519 suspected blood samples for Acute Febrile Illness (AFI) were collected in October/November 2019 (Monsoon Season). Whole blood was stored at 4°C and centrifuged at 1500 rpm for 10 minutes within 24 hours. Sera and plasma samples were stored at -20°C until use (12). All samples were tested for dengue and chikungunya IgM ELISA (kit supplied by NIV-Pune ). The diagnostic sensitivity and specificity of the kit is 95% and 98% respectively. Sera tested for presence of dengue IgM antibodies were from unknown patients and could be standard guidelines were followed for handling and disposing the tested sera. Biosafety norms of our laboratory have strictly followed. Contact with skin, mucous membrane and eyes with any of the reagents were avoided (13). All the test procedures were done according to literature as per manufacturer’s instructions. Optical density (OD) values were noted, cut-off values were calculated and results were documented. The demographic data of the patients included in the study population, name, gender sex and taluka of residence was documented (14).

II.2 PRINCIPLE OF THE TEST:

IgM antibodies in the patient’s serum are captured by antihuman IgM (μ chain specific) coated on to the solid surface (wells). In the next step, Dengue antigen is added which binds to captured human IgM in the sample. Unbound antigen is removed during the washing steps. In the subsequent step biotinylated flavivirus anti DEN monoclonal antibodies are added followed by avidin HRP. Subsequently, chromogenic substrate (TMB/H2SO4) is added, the reaction is stopped by 1N H2SO4. The intensity of colour/optical density is measured at 450nm (14&15).

II.3 Test Protocol: DENGUE IgM ELISA:

1) Select the sample to be tested write down the protocol on ELISA sheet provided with each kit.
2) Preparation of wash buffer: Dilute 20x wash buffer with distilled water for 1:20 concentration
3) Wash the ELISA micro well strip for 3 times and tap it to dry.
4) Add 50µl of negative and positive control in well A&B respectively.
5) Add 200 µl of sample diluent +2µl of sample to each well from C.(Incubate at 37°C,60min)
6) Wash the strips 5 times with prepared wash buffer and tap it to dry on tissue paper
7) Add 50µl of dengue antigen to each well (incubate at 37°C) repeat the step 6.
8) Add 50µl of dengue monoclonal antibody to each well (incubate at 37°C) repeat the step 6.
9) Add 50µl of avidin-HRP to each well (incubate at 37°C)
10) Add 100µl of TMB substrate to each well (incubate 10 min in dark at room temperature)
11) Add 100µl of stop solution to each well exact after 10mins.
12) Measure the absorbance at 450nm immediately (max with in 10mins)
III. RESULTS:

Samples of the patients with age group between 2 days to >75 years were collected, sex wise distribution revealed 519 samples which comprised of male 234 (45.1%) and female 285 (54.9%) (Fig-1). 114 positive for chikungunya IgM antibody, 22 positive were observed as coinfection (Fig-2). Sex wise distribution of positive samples are presented in (Fig-3). Amongst 234 samples collected from male patients, 29 (12.1%) samples reported positive for dengue IgM antibody, 29 (12.1%) samples reported positive for chikungunya IgM antibody and 9 (3.8%) samples reported coinfection for dengue and chikungunya. Similarly of the 285 collected from female patients, 29 samples were positive for dengue IgM antibody, 77 were positive for chikungunya and 13 reported positive for dengue and chikungunya coinfection. Age wise distribution of positive cases of both chikungunya, dengue and coinfection were studied. Distribution of positive patients was described starting from neonate to geriatric group with an interval of 10 years each (1 month to 70 years). The age group 0-1 year showed no positive cases for dengue infection, 1 positive case for chikungunya and coinfection each. Hence, this age group contributed least for presentation of positive cases. Age group 1-10 years reported 9 positive cases for dengue, 5 positive cases for chikungunya, and 5 diagnosed with coinfection. 11-20 years of age group presented maximum 14 cases of dengue infection, 19 chikungunya cases and 3 patients with coinfection. With 7 positive cases for dengue and 22 positive cases for chikungunya and 6 cases of dengue and chikungunya coinfection, 21-30 years of age group contributed for most positivity amongst all these age groups for dengue and chikungunya, coinfection and second most positive case for chikungunya infection. 31-40 age group is the group of people who are mostly outdoor workers, prone to external environment, hence this age group reported 4 positive cases for dengue infection and maximum of 23 chikungunya infection and 3 coinfection. Highest cases were reported in the above said age group. Whereas, the highest coinfection rate was observed at the age group of 21-30 (Table-1). The age group 41-50 presented only 1 positive case for dengue IgM and 18 positive cases for chikungunya IgM and only 1 for coinfection. Nil positive cases for dengue IgM at the age group of 51-60, 16 positive cases for chikungunya IgM and 2 for coinfection. Similarly, no case of dengue IgM was reported amongst 61-70 age group and 9 positive cases of chikungunya IgM was reported and only 1 was observed as coinfection. Category of patients above 70 years contributed only 1 case each for dengue and chikungunya IgM, no report of coinfection (Fig-10).

Fig-1: Sex Wise Sample Distribution.

Fig-2: Distribution of Positive Samples

Fig-3: Sex Wise Distribution of Positive Samples
Table 1: Age Wise Distribution of Positive Samples

<table>
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<tr>
<th>AGE</th>
<th>DENGUE IgM POSITIVE</th>
<th>CHIKUNGUNYA IgM POSITIVE</th>
<th>DEN IgM+CHIK IgM (coinfection)</th>
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</tr>
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<td>9</td>
<td>5</td>
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</table>

Fig 4: Age Wise Distribution of Positive Samples

Taluka wise distribution of samples collected and positive cases of dengue were documented. Samples collected from Kalaburgi urban (KLB-U) were 109 out of which 26 samples were positive. Out of 17 samples of Kalaburgi rural (KLB-R) only 1 sample found positive, though 8 samples were collected from Aland taluka there were no positive cases. From Afzalpur taluka 33 samples collected with only 1 positive case. From Chitapur taluka 139 samples collected which is highest number and the positive cases were 18. From Chincholli taluka 28 samples collected, 4 found positive for dengue. In Jewergi 1 positive case out of 9 samples was observed, 56 samples were collected from Sedam taluka and only 1 sample found to be positive for dengue monoinfection while 13 samples were received from other neighbouring districts and 6 samples reported positive (fig 5&6).

Similarly, Taluka wise distribution of samples collected and positive cases of chikungunya is documented in which, 107 samples collected from KLB-U and 31 cases found positive for chikungunya monoinfection. Of 20 samples collected from KLB-R with active surveillance, 7 cases were positive for chikungunya monoinfection. From Aland taluka 8 samples were collected in which 6 samples were found to be positive, Afzalpur reported just 1 positive case of 33 samples tested for chikungunya IgM. With highest 136 samples Chitapur contributed 28 positive cases for chikungunya monoinfection with active surveillance in several areas. Of the 28 samples received from Chincholli 6 samples reported positive for chikungunya IgM, Jewergi reported only 1 positive case of chikungunya monoinfection. Out of 9 samples collected, Sedam taluka reported highest 33 chikungunya monoinfection cases among 55 samples collected and 13 samples were collected from other neighbouring districts of which 4 reported positive for chikungunya monoinfection. Highest positivity is seen in Sedam with 33 positive cases for CHIK monoinfection followed by 31 positive cases from KLB-U and 28 positive cases from Chitapur taluka.

KLB-U presented the highest positivity rate with 26 dengue positive, 31 positive cases for chikungunya and 6 cases for dengue and chikungunya co-infection (Fig 7). Only one positive case for dengue was reported from KLB-R and 7 positive cases for chikungunya and no positive cases for coinfection. Aland taluka reported 6 positive cases of chikungunya and no case of dengue and coinfection. Afzalpur taluka also reported a single positive case for dengue and chikungunya and no positive cases for coinfection.
Chitapur taluka reported 18 positive cases for dengue IgM and 28 positive cases for chikungunya IgM and highest 8 positive cases for coinfection. Chitapur taluka reported second highest positivity rate for dengue and chikungunya and highest cases of coinfected among all other talukas. This taluka reported chikungunya outbreaks in some villages hence it contributed for most of the cases for all three infections. Chincholli taluka also reported 4 positive cases for dengue and 6 positive cases for chikungunya and 4 positive cases for coinfected. Jewergi taluka reported only 1 positive case for dengue, chikungunya and coinfection each. It is the least positivity reporting taluka after Afzalpur. Sedam taluka reported 1 positive case for dengue and 33 positive cases for chikungunya and 2 positive cases for coinfected. Several samples were received from other neighbouring districts, of them 6 reported positive for dengue IgM and 3 samples positive for chikungunya IgM and single positive case for coinfection (fig- 8 &9 ). Hence KLB-U reported maximum cases for dengue and chikungunya IgM and also for coinfection of dengue and chikungunya after Chitapur, which is second highest positivity rate for all three infections after KLB-U. Jewergi and Afzalpur are the talukas contributing least positivity rate among all three infections. Aland taluka contributed only 6 positive cases for chikungunya IgM and no positive cases for dengue and coinfection. In KLB-R, Aland,Afzalpur reported no positive cases for co infection of dengue and chikungunya. Samples from other districts showed minimum positivity rate for all the three infections. In contrast to other districts Sedam taluka reported only 3 positive cases for dengue and more positivity is observed for chikungunya monoinfection with 35 positive cases, since the positive
samples for dengue were only 3, the presentation of coinfection for dengue and chikungunya is also low with only 2 cases (Table-2).

Seasonal trends were observed from the monthly data collected from district surveillance unit Kalaburgi (DSU-klb) . Gradually, rise in the cases during the post monsoon period is observed, positive cases started rising from the month of August 2019 and declined in February 2020. A total of 519 samples were collected for the serological analysis of Acute Febrile Illness (AFI) (DENV & CHIKV) in which 285 samples were collected from female patients and 234 samples were from male patients (Fig-1). All samples were subjected to dengue and chikungunya IgM ELISA as per the standard protocol prescribed by the manual of NIV-Pune (42,43). It was observed that 114 (39.4%) samples were reported positive for chikungunya IgM , 58 (30.5%) samples were positive for dengue IgM and 22 (40.9%) samples were reported positive for coinfection (Fig-2). A month wise data collected from the district surveillance unit Kalaburagi regarding the month wise positive cases of dengue and chikungunya infections to analyse the seasonal trends in the emergence of these infection. Hence, the peak of coinfection could be analysed. In the month of January 2019, 22 and 24 positive cases for dengue and chikungunya IgM were observed. In the month of February 2019 positivity rate is similar to previous month with 23 and 14 positive cases for dengue and chikungunya respectively. In the month March the onset of summer season 22 positive cases of dengue infection and 16 positive cases of chikungunya were reported. The curve for dengue infection slightly elevated in the month of April 2019 with 45 cases and the curve for chikungunya infection remains same with respect to previous month with 13 cases. Regression in the positive curve was observed from the month of May to July 2019, comparatively with the previous months. In the month of May, 21 positive cases for dengue and 7 positive cases for chikungunya were reported. Statistics of June 2019 showed 18 positive cases for dengue and 8 positive cases for chikungunya. Slight elevation in the chikungunya is observed from the month of July with 26 positive cases but the dengue curve remained same with 17 cases. In the month of August 2019, 37 positive cases were observed and 24 positive cases for chikungunya. Sudden drop in the dengue infection is observed in the month of October 2019 with 8 cases and sudden elevation is observed in the chikungunya with 65 cases. Actual hike in the curve is observed from the October month in both the infection during post monsoon season which would have provided a better environment for the vector to breed. Highest positivity for chikungunya infection with 138 cases is observed in the month of October 2019 with 80 positive cases for dengue infection. Similarly, highest positivity for dengue infection is observed in November with 135 positive cases of dengue and 114 positive cases for chikungunya. Decline of positive cases was observed with regression in the curve for both the infections, from the month of December 2019 with 32 dengue and 37 positive cases of chikungunya (fig-10).
In our study 21-40 age group of patients presented maximum positive cases in all types of infections, this age group contributed for highest dengue positive cases. While, Mahinder Kaur et.al (17). Similar results were observed in the study conducted by karthik et.al (17). In our areas (26).

In our study 21-40 age group of patients presented maximum positive cases in all types of infections, this age group contributed 11(30.5%) out of 36 positive cases for dengue monoinfection, same age group reported 45(39.4%) out of 114 positive cases for chikungunya monoinfection, this age group presented the highest 9(40.9%) out of 22 positive cases for dengue and chikungunya coinfection. Hence, all types of monoinfection and coinfection is presented by this age group as this group is maximally involved in outdoor activities and being occupationally active and has higher chances of exposure to arboviruses. Similar, study trends was observed by age-wise distribution of the DENV-infected and CHIKV-infected samples revealed that majority of the patients were in the productive age group of 21–40 years which contributed for highest dengue positive cases. Our results correlated with Mahinder Kaur et.al (17).

Percentage distribution of dengue, chikungunya and coinfection, was compared with other reports which revealed, 58(29.8%) of dengue infection, 114(58.7%) of chikungunya infection and 22(11.3%) of coinfection in our study. While, Mahinder Kaur et.al (17) & Shireen et.al (12) reported 2178(68.92%) & 7(44%) respectively of dengue infection, 127(34.04%) & 9(29%) of chikungunya infection, while coinfection revealed by them is 27(9.54%) & 17(16%) respectively. These comparative studies have shown that, chikungunya infection and coinfection was more prevalent in our areas (26).
Lot of variation was observed when studies regarding dengue and chikungunya coinfection were compared. Rashmika Parmar (4) karthik R(18), Bhoosan S.Gandhi(19), Mahinder kaur(17), Modi K P(8), Htvi Chowda(11), Giriraj Vet.al(20), Nikhita Shrireenet.al(12) reported 205(7.6%), 25(6.8%), 20(6.7%), 27(9.54%), 43(3.9%), 60(3.7%), 38(9.5%), 17(16%) respectively of co-infection. While, our study reported 22(4.23%) which represents that out of 519 cases, 22(4.23%) was coinfection, while the above said report of 22(11.3%) coinfection was presented amongst 192 positive cases.

Current study of comparative distribution of dengue and chikungunya monoinfection particularly positive revealed 58(29.8%) dengue IgM positive and 114(58.7%) chikungunya IgM positive. Other authors reported 302(75.5%), 2178(68.2%), 992(17.15%), 474(12.44%), respectively of chikungunya IgM positive cases. Giriraja.v et.al. Maninder kaur et.al, Modi.p et.al, Htchipowda et.al(23,19,8,9).When these results were compared and correlated chikungunya monoinfection was predominant in our study while, dengue positive cases found to be medium.

Dengue and chikungunya are two important mosquito borne viral infections in India. Dengue virus and Chikungunya virus are transmitted by the same species of mosquitoes Aedes aegypti, Aedes albopictus. Both viruses cause acute febrile illness as infection progresses symptoms of both infection differ to the extent.

The incidence of dengue and chikungunya infection are increasing with the urbanization occurring in India. Cases of co-infection with dengue and chikungunya are becoming more prevalent in coming future due to increased transmission of both the viruses all over India. High attack rate in an immunologically naïve population showed cases infection progresses symptoms of both infection differ to the extent.

Co-infection with CHIKUNGUNYA virus is over shadowed by simultaneous occurrence of dengue fever. Dengue virus are transmitted by Aedes aegypti, Aedes albopictus while, dengue positive cases found to be medium.

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