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Original Article

Microbiology Section

Dengue and Chikungunya Mono and Co-infections among Patients with Acute Febrile Illness

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ABSTRACT

Introduction: Dengue and Chikungunya fever are arboviral diseases which are spread by a common vector. Being clinically indistinguishable, it is necessary to distinguish both either by molecular or serology testing.

Aim: To estimate the seroprevalence of Dengue and Chikungunya mono-infection as well as dual infection in patients with acute febrile illness.

Materials and Methods: Two hundred patients with acute febrile illness were enrolled from April 2015 to October 2016. Detailed clinical history was documented. Samples were collected and subjected to Polymerase Chain Reaction (PCR) and Enzyme Linked Immuno Sorbent Assay (ELISA) testing. For qualitative data, frequency percentage table was used and association was done using Chi-square test.

Results: Out of 200 patients, 8.5% had Chikungunya mono-infection and 41.5% patients had Dengue mono-infection. Dengue and Chikungunya co-infection was found in 4.5% patients. Most affected age group was 18-60 years wherein male preponderance was seen. In Chikungunya fever, 82.4% had morning stiffness and 35.3% had joint swelling; elbow and knee were the most commonly affected joints. In Chikungunya fever, 76.5% patients had restricted joint movements and 52.9% had Visual Analog Score (VAS) of 6-10. In Dengue fever, myalgia (67.5%) and rash (20.5%) were common symptoms. A total of 61.4% patients of Dengue fever had low platelet count. All Chikungunya cases and 88.1% Dengue cases detected by PCR had fever duration of less than five days. 85% of Chikungunya and 69% of Dengue cases detected by IgM ELISA had fever duration of more than five days.

Conclusion: Diagnostic algorithms of acute febrile illness cases should include testing by both molecular and serology for both the viruses, which is the absolute need of the hour.

Keywords: Dengue fever, Dual infection, Polymerase chain reaction

INTRODUCTION

In a tropical country like India, Dengue Virus (a Flavivirus) and Chikungunya Virus (an Alphavirus) are the common causes of arthropod borne acute febrile illness. Both these viruses are transmitted by the same Aedes species and have similar epidemiological pattern. Thus, it is expected to have co-infection with both the viruses [1]. The earliest case of Dengue Chikungunya co-infection was documented in Thailand [2]. More reports of co-infections were reported from India and Myanmar during periods 1964 to 1972 [1,3]. Subsequently, inspite of Dengue virus being in constant circulation in the endemic areas, Chikungunya-Dengue co-infections were not reported until the re-emergence of Chikungunya virus after 30 years in 2003 [4]. Beginning from 2006 until recently many co-infections have been reported from Africa, Mediterranean region, Pacific region and South East Asia including India [5]. In India, Chahar HS et al., detected 6 (8.7%) patients co-infected with Dengue and Chikungunya in 2006 in Delhi using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) [6]. By 2010, several cases of co-infection were reported from all over the country. Most of the co-infection studies were from Southern India [7-10]. A serological study from Karnataka showed dual infection varying between 5.7-9.5% [11].

Mumbai, a city with rapid urbanisation and humid climate, is endemic to Dengue infections [12]. The vector and natural reservoirs for both, Dengue and Chikungunya viruses are indigenous to Mumbai. This is compounded by prolonged wet season, low socio-economic conditions and high population density, thus contributing to the increased possibility of concurrent infections. There is no specific treatment for both the diseases. Supportive treatment is the main stay in case of Dengue fever, which if not initiated early, may lead to death in severe cases. However, in Chikungunya fever long lasting debilitating arthralgia can develop [4]. Thus, it is important to diagnose and distinguish Dengue fever from Chikungunya fever in order to monitor the long term effects of these infections. Both Dengue and Chikungunya have overlapping symptoms and hence may be difficult to distinguish clinically. Use of appropriate laboratory methods is mandatory for diagnosis. Often patients are tested only for Dengue virus and thereby Chikungunya infection goes unnoticed. Therefore, an accurate diagnosis cannot be made and estimates of burden and long term clinical effects are not recorded [13].

Chikungunya virus is known to present with the periodic waxing/waning pattern. This is probably due to exposed population being immunologically protected as there is only one infecting serotype. The disease again recurs in community when non-immune population becomes available after a gap of few years. Thus, it is important to have an insight on the current trends in the prevalence of Chikungunya infection and its co-infection with Dengue [14]. Inspite of the recent surge in cases of co-infection, there is still paucity of data on co-infections especially focusing on understanding the differences in clinical presentation in case of mono and dual infections. Few studies have used a psychometric response scale to measure intensity of joint pains and tried to differentiate between Dengue and Chikungunya infections [15,16]. Such screening tools if validated, will enable in early diagnosis, and monitoring during follow-up.

Therefore, considering above facts, the current study was designed to determine the seroprevalence of Dengue and Chikungunya infection in an urban city of western Maharashtra along with its clinical consequences.

MATERIALS AND METHODS

The prospective study was conducted in Department of Microbiology, at TNMC and BYL Nair Charitable Hospital, Mumbai, Maharashtra,

India, from April 2015 to October 2016. The study was approved by the Ethics Committee for Academic Research Projects (ECARP), PG Academic Committee, TN Medical College and BYL Nair Charitable Hospital, Mumbai, Maharashtra, India, (Ethics approval number: ECARP/2015/17, dated 23rd April, 2015) and informed consent was obtained from each participant. Patients above 18 years of age with clinical suspicion of Dengue and Chikungunya fever presenting with acute febrile illness and joint pain/myalgia were included in the study. Patient's positive for malaria, leptospirosis and typhoid fever were excluded from the study.

Sample size was calculated with 98% confidence interval using prevalence rate of Dengue Chikungunya co-infection (10%) from previous studies [15-17]. Detailed history of each patient was taken at a single time point. Demographical data as well as clinical findings pertinent to joint involvement were recorded. VAS for severity of joint pain was also documented. Haematological investigations were performed as routine. All data was collected at single point of time and no patient follow-up was done.

Five mL of blood was collected in plain and EDTA vacutainer each. Serum and plasma were separated respectively. Serum was tested for both Dengue IgM Antibody and Chikungunya IgM Antibody by ELISA method. Plasma was tested for Dengue and/or Chikungunya virus by RT-PCR. As per Centers for Disease Control and Prevention (CDC), a laboratory confirmed case of Dengue or Chikungunya is defined when either IgM ELISA or RT-PCR is tested positive for respective viruses [18]. ELISA kit for Dengue and Chikungunya IgM ELISA was obtained from National Institute of Virology, Pune. For RT-PCR, nucleic acid extraction was done using Spinstar TM Viral Nucleic Acid Kit 1.0 (ADT Biotech, Malaysia). Multiplex real time PCR was done using FTD Dengue/Chik PCR kit (manufactured by Fast Track Diagnostics, Luxembourg). Amplification was performed in the ABI 7500 real-time PCR system thermocycler (Applied biosystems, USA). All the tests were performed as per the respective kit literature.

STATISTICAL ANALYSIS

Quantitative data was presented as mean and comparison was done by Student's unpaired t-test. Qualitative data was presented as frequency percentage and association among groups was done with help of Chi-square test. A p-value of <0.05 was taken as statistically significant.

RESULTS

Majority of the patients with Chikungunya fever (94.1%), Dengue fever (96.4%) and co-infection (88.9%) were found in the age group of 18-60 years. Overall, male preponderance was observed [Table/Fig-1].

Age in years	Chikungunya fever (n=17)	Dengue fever (n=83)	Co-infection (n=9)
	No (%)	No (%)	No (%)
18-30	**6 (35.28)**	**63 (75.9)**	**4 (44.44)**
Male	3 (17.64)	34 (40.96)	3 (33.33)
Female	3 (17.64)	29 (34.94)	1 (11.11)
31-45	**9 (52.93)**	**15 (18.06)**	**4 (44.44)**
Male	6 (35.28)	9 (10.84)	3 (33.33)
Female	3 (17.64)	6 (7.22)	1 (11.11)
46-60	**1 (5.88)**	**2 (2.40)**	**0 (0)**
Male	1 (5.88)	1 (1.2)	0 (0)
Female	0 (0)	1 (1.2)	0 (0)
>60	**1 (5.88)**	**3 (3.6)**	**1 (11.11)**
Male	1 (5.88)	2 (2.4)	0 (0)
Female	0 (0)	1 (1.2)	1 (11.11)

[Table/Fig-1]: Distribution of age and gender in suspected Dengue and Chikungunya cases.

Out of 200 cases, 17 (8.5%) were Chikungunya virus mono-infected. Among these, 4 (2%) cases were detected by RT-PCR and 13 (6.5%) cases were identified to have Chikungunya IgM antibody by ELISA. Dengue virus mono-infection was seen in 83 (41.5%) patients, out of which, 48 (57.8%) were positive by RT PCR whereas 26 (31.3%) cases had Dengue IgM antibody as determined by ELISA. Dengue and Chikungunya co-infection was observed in 9 (4.5%) patients with IgM antibodies to both viruses present in 7 (3.5%) cases. A total of 91 patients were tested negative for dengue and chikungunya by both IgM ELISA and RT-PCR [Table/Fig-2].

	Chikungunya mono-infection	Dengue mono-infection	Chikungunya and Dengue co-infection
Positive by ELISA	13	26	7
Positive by RT-PCR	4	48	2
Positive by both tests	0	9	0
Total	**17 (8.5%)**	**83 (41.5%)**	**9 (4.5%)**

[Table/Fig-2]: Results of Dengue and Chikungunya IgM ELISA and RT-PCR (n=200).

Fever and joint pain was present in all of the 200 patients enrolled in this study. Among patients with Chikungunya mono-infection, morning stiffness (82.4%) was most common symptom. Joint swelling (p=0.02) and morning stiffness (p<0.001) was seen significantly more in patients with Chikungunya fever than in Dengue fever [Table/Fig-3].

Symptoms	Chikungunya fever		Dengue fever		Co-infection	
	No (%)	p-value	No (%)	p-value	No (%)	p-value
Fever	17 (100)	-	83 (100)	-	9 (100)	-
Joint pain	17 (100)	-	83 (100)	-	9 (100)	-
Myalgia	11 (64.7)	0.65	56 (67.5)	0.59	5 (55.6)	0.35
Headache	10 (58.8)	0.74	22 (26.5)	**<0.001**	4 (44.4)	0.14
Joint swelling	6 (35.3)	**0.02**	13 (15.7)	0.78	1 (11.1)	0.71
Morning stiffness	14 (82.4)	**<0.0001**	8 (9.6)	0.10	2 (22.2)	0.36
Rash	2 (11.8)	0.80	17 (20.5)	**<0.0001**	1 (11.1)	0.91
Total (n)	**17 (100)**		**83 (100)**		**9 (100)**	

[Table/Fig-3]: Distribution of symptoms in suspected Dengue and Chikungunya cases. p-value calculated using Chi-square test with statistical significance when p<0.05.

Rashes were seen in significantly more number in patients with Dengue fever (20.5%) as compared to patients with Chikungunya fever (11.8%) and co-infection (11.1%). In patients with co-infection, myalgia (55.6%) and headache (44.4%) were common symptoms [Table/Fig-3].

In Dengue fever, 20.5% patients had involvement of small joints as compared to 11.1% in co-infection. None of the patients with Chikungunya fever had small joint involvement. This was statistically significant with p=0.02 [Table/Fig-4].

Type of joint	Chikungunya fever (n=17)		Dengue fever (n=83)		Co-infection (n=9)	
	No (%)	p-value	No (%)	p-value	No (%)	p-value
Knee	13 (76.5)	0.57	72 (86.7)	0.10	8 (88.9)	0.55
Elbow	14 (82.4)	0.72	69 (83.1)	0.22	7 (77.8)	0.92
Ankle	12 (70.6)	0.49	54 (65.1)	0.61	5 (55.6)	0.63
Small joints	0 (0)	0.10	17 (20.5)	**0.02**	1 (11.1)	0.71

[Table/Fig-4]: Distribution of joint involvement in suspected Dengue and Chikungunya cases.
p-value calculated using Chi-square test with statistical significance when p<0.05

VAS of 6-10 was observed in 52.9% of patients with Chikungunya fever and 55.6% of patients with co-infections as compared to only 31.3% of patients with Dengue fever. This was statistically significant (p=0.02) [Table/Fig-5].

A total 13/17 (76.5%) patients of Chikungunya fever had restricted joint mobility as compared to 15/83 (18%) in dengue fever and 1/9 (11.1%) in co-infection (p<0.0001) [Table/Fig-6].

VAS score	1-5	6-10	Total	p-value
Chikungunya fever (n=17)	8 (47.1)	9 (52.9)	17 (100)	**0.02**
Dengue fever (n=83)	57 (68.7)	26 (31.3)	83 (100)	0.54
Co-infection (n=9)	4 (44.4)	5 (55.6)	9 (100)	0.07

[Table/Fig-5]: Distribution of VAS Score in suspected Dengue and Chikungunya cases.
p-value calculated using Chi-square test with statistical significance when p<0.05

Joint movement	Restricted	Non restricted	Total	p-value
Chikungunya fever (n=17)	13 (76.5)	4 (23.5)	17 (100)	**<0.0001**
Dengue fever (n=83)	15 (18)	68 (82)	83 (100)	0.44
Co-infection (n=9)	1 (11.1)	8 (88.9)	9 (100)	0.70

[Table/Fig-6]: Distribution of restriction of joint movement.
p-value calculated using Chi-square test with statistical significance when p<0.05

Haematological analysis showed, 82.4% patients with Chikungunya fever, 63.9% patients with Dengue fever and 66.7% patients with co-infection had normal haemoglobin. Majority of patients had normal white blood cell count.

In Chikungunya fever and co-infection, 13/17 (76.5%) and 2/9 (22.2%) patients had normal platelet count. In Dengue fever, 51/83 (61.4%) patients had low platelet count of less than 1.5 lac/cmm. Of these, 9/33 (27.3%) patients had platelet count of less than 50000/cmm. In Dengue fever significantly higher number of patients had low platelet count as compared to patients with Chikungunya fever. This was statistically significant (p=0.004) [Table/Fig-7].

Range/cmm		Chikungunya fever	Dengue fever	Co-infection
		No (%)	No (%)	No (%)
Low platelet count (<150000)	<20,000	0 (0)	3 (3.6)	0 (0)
	20,000-50,000	0 (0)	6 (7.2)	1 (11.1)
	50,000-1,00,000	1 (5.9)	24 (28.9)	4 (44.4)
	1,00,000-1,50,000	3 (17.6)	18 (21.6)	2 (22.2)
Normal platelet count (>150000)		13 (76.5)	32 (38.6)	2 (22.2)
Total (n)		**17 (100)**	**83 (100)**	**9 (100)**

[Table/Fig-7]: Distribution of platelet count in suspected Dengue and Chikungunya cases.

Fever of less than five days of duration was seen in 7/17 patients (41.2%) with Chikungunya fever and 2/9 patients (22.2%) with co-infection. In Dengue fever, 66.3% patients presented with fever less than five days and 33.7% presented with fever more than five days of fever [Table/Fig-8].

Fever days	Chikungunya fever	Dengue fever	Co-infection
	No (%)	No (%)	No (%)
<5	7 (41.2)	55 (66.3)	2 (22.2)
≥5	10 (58.8)	28 (33.7)	7 (77.8)
Total (n)	**17 (100)**	**83 (100)**	**9 (100)**

[Table/Fig-8]: Distribution of fever duration in suspected Dengue and Chikungunya cases.

Among patients positive for Chikungunya IgM antibody by ELISA, 17/20 (85%) had fever duration of more than five days, whereas 29/42 (69.0%) patients positive for Dengue IgM antibody by ELISA had fever duration of more than five days.

All patients (6/6) positive for Chikungunya RT-PCR had fever duration of less than five days and 52/59 (88.1%) positive patients for Dengue RT-PCR had fever of less than five days [Table/Fig-9].

Fever duration in days	Chikungunya fever		Dengue fever	
	IgM ELISA	RT-PCR	IgM ELISA	RT-PCR
	No (%)	No (%)	No (%)	No (%)
<5	3 (15)	6 (100)	13 (30.9)	52 (88.1)
≥5	17 (85)	0 (0)	29 (69.0)	7 (11.9)
Total (n)	**20**	**6**	**42**	**59**

[Table/Fig-9]: Distribution of duration of fever and positivity in IgM ELISA and RT-PCR test.

DISCUSSION

Chikungunya virus re-emerged in Indian sub-continent as an explosive epidemic in 2005. However, by 2011, the overall incidence of Chikungunya infection in India started showing the downward trend [19]. This may be due to the development of herd immunity. The present study was undertaken to estimate the percentage distribution of Chikungunya and Dengue, mono and dual infections. In the present study, 26/200 (13%) of cases had IgM antibody against Dengue virus alone whereas 13/200 (6.5%) cases had IgM antibody against Chikungunya virus only. A total of 7/200 (3.5%) cases had IgM antibodies against both viruses simultaneously. There are limited studies, mostly serological, reporting the co-infections from the Western part of India. Gandhi BS et al., identified dual infection with Chikungunya and Dengue viruses in 25/364 (6.8%) of the patients by IgM ELISA from Pune [20]. In another Mumbai based study by Galate LB et al., 75.5% patients had only Dengue antibodies, 3% patients had only Chikungunya antibodies and 9.5% patients had antibodies for both Chikungunya and Dengue viruses [15]. In a recent study from Mumbai by Londhey V et al., a total of 20/300 (6.7%) of co-infection was detected by ELISA testing and 30/300 (10%) by RT-PCR testing [16].

Age

In 2011-12, Mohanty I et al., from Southern Odisha have also reported 54.5% of Chikungunya cases in the age group 16-45 years [21]. Londhey V et al., have reported 50.3% cases of Chikungunya fever in the age group of 31-45 years from Mumbai in 2010-2015 which conforms with this study, in which majority of cases (88.2%) of Chikungunya fever were in age group of 18-45 years [16]. In the present study, it was found that patients with Dengue fever, 94% patients were in age group of 18-45 years. Three cases were in the geriatric age group. 58.5% Dengue cases were reported by Das SS et al., in the age group of 31-45 years from Mumbai in 2013 [22]. In 2012-2013, Galate LB et al., reported 50.98% cases of Dengue infection in the age group of 31-45 years from Mumbai [15]. In current study, 88.9% patients with Dengue and Chikungunya co-infection were in age group of 18-45 years. One case was identified in the geriatric age group. Galate LB et al., reported 63.15% cases of co-infection in the age group of 31-45 years from Mumbai in 2012-2013 [15]. In a study in 2016 from Amritsar by Kaur M et al., highest number of co-infected cases was found in the age group of 21-40 years [23].

Gender

In the present study, male preponderance was seen in 64.7% in patients with Chikungunya mono-infection, 55.4% in patients with Dengue mono-infection, 66.6% in patients with co-infection. Gandhi BS et al., reported similar findings from Pune in 2010 with 59% males with Chikungunya infection, 67% males with Dengue infection and 68% males with co-infection [20]. Male preponderance was also reported by Dinkar A et al., and Singh J et al., [24,25]. Males are more involved in outdoor activities and hence, more exposed to the mosquito bites. Thus, male preponderance was seen.

Symptoms

In present study, significantly more number of patients with Chikungunya fever had joint swelling (35.3%) and morning stiffness as compared to patients with only Dengue infection and patients with co-infection (p=0.02). In a study conducted by Galate LB et al., in 2012-13 at Mumbai, fever and joint pain were most frequently observed symptoms while other symptoms were myalgia (66.6%), headache (42.1%) and joint swelling (26.3%) in patients co-infected

with Chikungunya and Dengue viruses [15]. In this study, rash was found in significantly more number of patients with Dengue fever than in patients with Chikungunya or co-infection (p<0.0001). In a study from Hyderabad by Neeraja M et al., fever (100%) with joint pains/ myalgia (32%) was predominant symptoms while rash (31%) and headache (26%) were other prominent findings of Dengue fever [8].

Polyarthritis

The polyarthralgia in Chikungunya fever may involve not only small joints like ankle, wrist but also large joints such as knee and shoulder [26]. In the present study, elbow joint followed by the knee joint were the most commonly involved joints in all the groups. This highlights the larger joint involvement in Chikungunya fever. In the study from North India by Mehta KD al., knee joint (92.5%) was the most commonly involved joint followed by ankle joint (70%) and elbow joint (55%) in patients with Chikungunya fever [27].

In the present study, 76.5% patients of Chikungunya fever, showed restricted joint mobility as compared to one patient with co-infection and 18% patients of Dengue fever. Thus in this study significant association was observed between restriction in joint mobility in patients with Chikungunya mono-infection as compared to patients with co-infection and only Dengue fever. Galate LB et al., from Mumbai have also reported restriction in joint mobility in all patients with Chikungunya fever [15]. Only 5.26% of patients with co-infection and 17.88% of patients with Dengue fever had restricted joint mobility.

Fever Duration

Viral genome can be detected by RT-PCR in blood until 5th day of fever. After day 5, virus disappears and antibodies start appearing which can be tested by IgM ELISA [26-28]. In the present study, 85% patient's positive for Chikungunya IgM antibody by ELISA and 69.0% patient's positive for Dengue IgM antibody by ELISA had fever duration of more than five days. As IgM antibodies usually become detectable by five days of fever, these findings can be co-related.

In current study, six patients were positive for Chikungunya RT-PCR. All these 6 patients (100%) had fever duration of less than five days. 52 (88.1%) patients who were positive for Dengue RT-PCR had fever duration of less than five days. Thus, RT-PCR assay was most sensitive during first five days of fever and declines after 5th day where upon IgM antibodies can be detected. This finding was confirmed by Ray P et al., in a multi-centric study conducted in 2009 [29].

Haematological Findings

Only 38.6% patients with Dengue mono-infection and 22.2% patients with co-infection showed normal platelet counts. Hence, statistically significant number of patients with chikungunya mono-infection have normal platelet count as compared to patients with co-infection and Dengue infection. In the study conducted by Murhekar MV et al., at Tirunelveli in 2009-10, majority of Chikungunya fever patients had normal platelet count [30].

Detecting and differentiating Dengue fever and Chikungunya fever pose a challenge because of the mimicking clinical presentation. Thus, in endemic areas, testing for both the viruses should be done. A proper laboratory based surveillance is also important to determine and establish the epidemiological pattern of chikungunya and its co-infection with dengue. Diagnostic kits for detecting both viruses simultaneously are challenging. As there is cross-reactivity between the sero-complexes of both Dengue and Chikungunya virus, differentiation by serology is limited and has to be used with utmost caution [31]. Without the reliable serological method for the early detection, molecular methods such as RT-PCR with high sensitivity hold promise. Saha K et al., developed a One-step Duplex RT-PCR which is rapid and differentiates Dengue and Chikungunya virus efficiently [32]. These one step RT-PCR do not cross react and have low rate of contamination. Another method of viral nucleic acid detection is Loop Medicated Isothermal Amplification (LAMP) which is carried out at single temperature with rapidity and comparable sensitivity [33].

Recently, Jain J et al., tested the efficacy of an Immuno-Chromatography (IC) card test using viral envelope protein in detecting Chikungunya virus [34]. Authors found that the detection rate of the IC kit was comparable to RT-PCR but much better than that of the IgM ELISA. However, the detection rate decreases beyond the first week of illness. Further research in this area is the need of the hour.

Limitation(s)

The limitation of the present study was the small sample size.

CONCLUSION(S)

Dengue and Chikungunya virus circulate and can infect simultaneously. VAS scoring is easy and useful tool in differentiating arthralgia due to Chikungunya virus and Dengue Virus. In initial days of fever, RT-PCR testing is more beneficial whereas utility of IgM ELISA testing is after five days of fever onset. Need of the hour is that the diagnostic algorithm of acute febrile illness cases should include testing for both the viruses.

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