Chikungunya and Dengue Co-Infection: Emerging Insight Threat

Jyotsna Chandwani1*, Geeta Parihar2, Shailendra Garg3, Lakshya Sharma4

1*Senior Resident, 2Senior Professor, 3Assistant Professor, Department of Microbiology, J.L.N Medical College & Hospital, Ajmer, Rajasthan, India. 4BSc Microbiology, Jaipur Engineering College & Research Centre, Jaipur, Rajasthan, India.

ABSTRACT
Background: Chikungunya virus (CHIKV) and Dengue virus (DENV) are arboviruses transmitted to humans by hematophagous arthropods like Aedes aegypti mosquito. As these diseases cause several clinical symptoms, they have become an emerging global public health threat. Co-circulation of CHIKV and DENV is increasing around the world and must therefore be considered as a threat. There are a few studies discussing the Chikungunya-Dengue co-infection from Western India. The present project was undertaken to study the magnitude of the two diseases, presence of co-infection & other factors.

Material & Method: This was a retrospective laboratory based study, conducted in J.L.N Medical College & Hospital Ajmer, Rajasthan, India. A total number of 319 patients of all age group having acute febrile illness with clinical features attending OPD & IPD, from September 2016 to February 2017 were taken in this study. The serological diagnosis was done by IgM captured ELISA. Statistical analysis was performed with the SPSS, Trial version 23 for Windows statistical software package and Primer.

Results: During the study duration, a total of 319 cases were studied, with the mean age of 27.51 ±14.54 years. The maximum proportion of the cases (76.81% of the total cases) was observed in the month of October–November. Out of the total, 53.20% was males. Co infection was observed in 22.57% of the cases while mono infection was observed in 77.43%. Among them 113 /247 (45.57%) cases were Dengue and rest suffered with Chikungunya 134/247(54.25%).

Conclusion: CHIKV and DENV are transmitted by Aedes mosquito species and they cause severe, several clinical symptoms in common. As their dual infection cause increase morbidity and mortality & dual infection is on verge of increase, the early and timely diagnosis of both viruses should be made mandatory for appropriate management of patient.

Key words: Aedes aegypti, Chikungunya, Dengue.

*Correspondence to:
Dr. Jyotsna Chandwani,
Senior Resident,
Department of Microbiology,
J.L N Medical College & Hospital,
Ajmer, Rajasthan, India.

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INTRODUCTION
Arthropod-borne viruses represent a global threat for public health as they can be transmitted to humans by hematophagous arthropods that are rapidly spreading worldwide. Both Chikungunya (CHIK) and Dengue (DEN) fever are one of the important emerging arboviral infections. Both the viruses are transmitted to humans by hematophagous arthropods like Aedes aegypti mosquito. As they share the common vector, their co-infections in the same geographical areas, are common & are on the verge of increase.1,2

Chikungunya virus (CHIKV) belongs to the family Togaviridae. It is an icosahedral-shaped, single-stranded, positive sense, enveloped RNA virus; its genome is approximately 12 kb. Clinically, the infection is characterized by fever, rash, fatigue, headache, intense body aches & often persistent arthralgia, which last for years in some individuals. It was first documented in 1952 in Africa. The virus dramatically re-emerged in Kenya in 2004 with massive sporadic CHIKV outbreaks in countries of Africa and south-eastern Asia (India was most affected country with more than 1.4 million infections).1,3

Dengue fever is also called break-bone fever and is caused by dengue virus (DENV) belonging to family Flaviviridae. It is a single stranded, positive-sense, enveloped RNA virus having genome of 10.6 kb in length. DENV has four serotypes (DENV 1-4), which only have transient cross protection with each other. Dengue fever is characterised by saddleback fever, severe headache, muscle, joint pain, vomiting, eye pain and rash. Its severe forms are dengue hemorrhagic fever and dengue shock syndrome.1,3

According to the WHO reports ,more than 125 countries and recently 390 million infections occur annually. Case fatality rates is between 0.5 % – 3.5 %.
As they share the common vector, their co-infections in the same geographical areas are common & are on the verge of increase. Co-circulation of CHIKV and DENV is increasing around the world and must therefore be considered as a threat which should be taken seriously. There are few studies discussing the Chikungunya-Dengue co-infection from Western India. The present project was undertaken to study the magnitude of the two diseases, presence of co-infection, seasonal variation & also know the association of age and gender.

MATERIALS AND METHODS
This was a laboratory based observational, descriptive & retrospective type of study, conducted in J.L.N Medical College & Hospital Ajmer, Rajasthan, India. A total number of 319 patients of all age groups, having acute febrile illness with clinical features of Chikungunya and Dengue fever attending OPD & IPD, from September 2016 to February 2017, were taken in this study. The serological diagnosis was done by IgM capture enzyme-linked immunosorbent assay (ELISA) from NIV, Pune. All the samples were subjected to an ELISA test to detect the presence of immunoglobulin M (IgM) antibodies against both CHIKV and DENV, by IgM antibody-capture (MAC)-ELISA kits (Arboviruses Diagnostic NIV, Pune, India). The sensitivity and specificity for the CHIK IgM antibody capture ELISA is 95 % and 97.22%, respectively, and for dengue IgM antibody capture ELISA is 98.53% and 98.84%, respectively. The tests were carried out following the manufacturer’s instructions.

Principle of IgM Capture ELISA for CHIKV and DENV
IgM antibodies in the patient’s blood are captured by antihuman IgM (μ chain specific) that is coated on to the solid surface (wells). In the next step, CHIK/DEN antigen is added, which binds to captured IgM, if the IgM and antigen are homologous. Unbound antigen is removed during the washing step. In the subsequent steps Biotinylated anti CHIK/DEN monoclonal antibody is added followed by Avidin-Horse radish peroxidase (HRP). Subsequently, substrate/ chromogen, Tetramethylbenzidine (TMB) - H2O2 is added and monitored for development of colour. The reaction is stopped by 1NH2SO4. The intensity of colour/optical density is measured at 450 nm.

Statistical analysis: Statistical analysis was performed with the SPSS, Trial version 23 for Windows statistical software package (SPSS inc., Chicago, il, USA) and Primer. The Categorical data was presented as numbers (%) and were compared among groups using Chi square test. The quantitative data was presented as mean and standard deviation and were compared by students, using, t-test, ANOVA Test and post Hoc Tukey’s Test applying to find out the most significant groups among all the groups. P value <0.05 was considered statistically as significant.

Table 1: Demographic characteristic of the study population

<table>
<thead>
<tr>
<th>No of cases</th>
<th>319</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±SD age</td>
<td>27.51 ± 14.54</td>
</tr>
<tr>
<td>Month</td>
<td>Number</td>
</tr>
<tr>
<td>SEPTEMBER (2016)</td>
<td>29</td>
</tr>
<tr>
<td>OCTOBER (2016)</td>
<td>114</td>
</tr>
<tr>
<td>NOVEMBER (2016)</td>
<td>131</td>
</tr>
<tr>
<td>DECEMBER (2016)</td>
<td>31</td>
</tr>
<tr>
<td>JANUARY (2017)</td>
<td>8</td>
</tr>
<tr>
<td>FEBRUARY (2017)</td>
<td>6</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>FEMALE</td>
<td>147</td>
</tr>
<tr>
<td>MALE</td>
<td>172</td>
</tr>
</tbody>
</table>

Fig 1: Seasonal variation of the infection
Table 2: Association of age with type of infection

<table>
<thead>
<tr>
<th>Diseases</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P Value LS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHIK+</td>
<td>134</td>
<td>33.83</td>
<td>14.99</td>
<td></td>
</tr>
<tr>
<td>DEN+</td>
<td>113</td>
<td>19.89</td>
<td>8.91</td>
<td></td>
</tr>
<tr>
<td>Co-Inf+</td>
<td>72</td>
<td>27.71</td>
<td>15.29</td>
<td>&lt;0.001S</td>
</tr>
<tr>
<td>Mono infection</td>
<td>247</td>
<td>27.45</td>
<td>14.35</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>319</td>
<td>27.51</td>
<td>14.54</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Association of gender with type of infection

<table>
<thead>
<tr>
<th>GENDER</th>
<th>CHIK+</th>
<th>DEN+</th>
<th>Co-Inf+</th>
<th>Mono</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEMALE</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>67</td>
<td>50.0</td>
<td>48</td>
<td>42.48</td>
<td>32</td>
</tr>
<tr>
<td>MALE</td>
<td>67</td>
<td>50.0</td>
<td>65</td>
<td>57.52</td>
</tr>
<tr>
<td>Total</td>
<td>134</td>
<td>113</td>
<td>72</td>
<td>247</td>
</tr>
</tbody>
</table>

RESULTS

During the study duration total 319 cases was studied, with the mean age was 27.51 ±14.54 years. Most of the cases were in age group 21 to 40 years (41.69%). The maximum proportion of the cases (76.81%) was observed in month of October –November. Out of the total 53.20% were males. (Table 1 & Fig 1) Co infection was observed in 22.57% of the cases while mono infection was observed in 77.43%. Among them 113 /247 (45.57%) cases were Dengue and rest suffered with Chikungunya 134/247(54.25%). (Fig 2)

Association of age with type of infection was observed statistically significant. (P<0.001S) .Significantly higher mean age was observed in Chikungunya cases as 33.38±14.09 years as compared to dengue 19.89 ±8.91. (Table 2)

No significant difference was observed according to sex with the type of infection .(P=0.83NS), although the males were more affected as compared to females as (57.52% vs. 42.48% ) in dengue cases and in Chikungunya there were equal cases . Also in mono infection & co-infection males were more in comparison to female. (Table 3)

DISCUSSION

In Calcutta (1963) during the Dengue Haemorrhagic Fever outbreak, DENV and CHKV were found circulating together. After about three decades, CHIK outbreaks with sporadic cases of dengue were reported from different areas of India. Co infection with DENV and CHIKV is dreadful, and is on verge of increase globally, especially in India.7 In our study, 319 cases were included, out of which most of the cases mean age was 27.51 years. Our finding was similar to the study done by Nazia Afreen et al (2014) in which it was 25.67 years.

Among the total cases males (53.20%) superseded the females (46.80%); Beatrice Chipwaza et al (2014) also reported 51.1% males which was consistent with our finding.9 Maximum proportion of cases was observed in between October -November month (76.81%) which comparable to Bhooshan S. Gandhi et al (2015) and many studies, as post monsoon period the breeding of mosquito increases.10

Mono infected cases were 77.47%, which included 42.01 of 35.42% Chikungunya and dengue respectively, our results were comparable with a study done by Modi.K.P et al(2017) in which
Chikungunya and dengue were 33.61% & 20.26% respectively. Dengue and Chikungunya co-infected cases were 22.57% compared to the study done by Karthik et al (2013) & Kalawat et al. (2011) in which the occurrence was 7.60% & 2.70% respectively, which clearly indicates the emergence of co-infection is increasing.

In our study association of age with type of infection was observed statistically significant. (P<0.001S) It was observed that mean age was higher in Chikungunya cases as 33.38 years as compared to dengue (19.89 years). Nazia Afreen et al also echoed the same finding in which mean age of Chikungunya and dengue were 31.67 years & 24.69 years. However, a study done by Kularatne SA et al reported a higher mean age which was 45 years & 30 years respectively. The mean age of co-infected cases was 27.71 years, which was comparable to Nazia Afreen et al (22.8 years).

No significant difference was observed that according to sex with type of infection. Seasonal variation was observed with all kinds of infections. There was rise of the cases during the post monsoon period i.e. between October—November, Kalawat et al also reported the same. As an increase in co-infection trend was observed in this study, it is a need that, the physician should be alert & vigilant in clinical perspective, diagnosis & complications.

CONCLUSION
CHIKV and DENV are transmitted by Aedes mosquito species and they cause several, severe, dreadful clinical symptoms in common. The cases of co infection with both viruses have become common and are on the verge of an increase in the future especially in countries like India. Their dual infection cause increases morbidity and mortality. The early and timely diagnosis of both viruses should be made mandatory for appropriate management of patient. Our study will help in the recognition of CHIKV/DENV co-infections and suggests that the clinicians should advice tests to detect the presence of both viruses in individuals showing clinical features of infection with either of the two.

REFERENCES
6. National Institute Of Virology: www.niv.co.in

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Conflict of Interest: None Declared.
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