

Chikungunya Confused With Dengue In Malaysia :Clinical, Serological And Molecular Perspective

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Citation

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Abstract

This study was to observe clinical, serological and molecular diagnosis of chikungunya viral fever patients and its comparison with dengue viral fever. For that 49 serologically negative dengue patients but clinically dengue like symptoms were included. Clinical investigation was carried out recording different profiles of hospitalized patients. Serology was performed using the Onsite® Chikungunya IgM Combo rapid test and molecular test RT-PCR was performed to detect the virus in the patients sera. It was observed that out of them 19 (38.7 %) were serologically confirmed chikungunya infection. Interestingly molecular detection of the patients sera did not show the presence chikungunya virus but detected dengue virus from 9 patients's sera. Classical clinical features of chikungunya virus infected patients were recorded to differentiate chikungunya from dengue, which were fever, arthralgia, myalgia and rash. Detection of chikungunya IgM in these cohort means that this virus is circulating throughout the year although not as many as dengue. Laboratory confirmation is important to differentiate chikungunya fever from dengue fever. This study adds some information in local data and hopefully can help the clinicians to clinically and laboratory diagnosis and management of chikungunya infection in outbreak and non-outbreak setting. Serology was proven to be useful in confirming chikungunya infection.

INTRODUCTION

Chikungunya virus (CHIKV) infection has attracted so much attention since 2005. During that time it caused chikungunya fever with epidemics reported in Asia and Africa. Chikungunya is a crippling disease caused by the virus belong to the family *Togaviridae* (Chakkaravarthy et al, 2011). It is an arbovirus that shares the same vector with dengue virus. Thus, in dengue-endemic region, chikungunya is also a significant cause of viral fever causing outbreaks associated with severe morbidity. Clinically the virus causes high grade fever, chills and rigors associated with severe arthralgia and myalgia (Venkatesan et al, 2010) In Asia, urban mosquito sp, *Aedes aegypti* are primary vectors (El-Badry and Al-Ali, 2010) in comparison to forest-dwelling *Aedes* spp. seen in Africa. These spp are urban and peridomestic, anthropophilic mosquitoes that maintain close associations with humans and thus, are likely responsible for regional large outbreaks (Powers and Logue 2007).

Since April 2008, nationwide outbreak has affected Malaysia with 4165 cases in 2008. In 2009, the Ministry of Health in Malaysia reported over 5430 cases of chikungunya fever. The most affected areas were the northern provinces of Sarawak, Kedah, followed by Kelantan, Selangor, and Perak.

Chikungunya viral activity continued in the year 2010, with an additional 325 cases reported in the first 5 weeks (MOH, 2010).

The symptoms of CHIKV infection are quite similar to those caused by many other infectious agents in the endemic areas. One particular difficulty in identifying CHIKV infection is its overlapping distribution with dengue viruses. It has been postulated that many cases of dengue virus infection are misdiagnosed and that the incidence of CHIKV infection is much higher than reported (Carey 1971). As a result management of patients with CHIKV infection has not been taken care of focusing appropriate causal agent. Through study has not been undertaken to determine the clear picture of CHIKV infection and its comparison with dengue in respect of clinical and serological and molecular investigation. Though, clinical and laboratory diagnosis are important for overall management and control of the disease. Therefore, the present study was undertaken to diagnose chikungunya infection by clinical, serological and molecular method in clinically suspected dengue patients presented to University Kebangsaan Malaysia Medical Centre (UKMMC).

MATERIAL AND METHODS

Study area: This study was conducted during January 2009 to January 2010 at Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Faculty of Medicine, National University Malaysia, Cheras-56000, Kuala Lumpur, Malaysia. The UKMMC has 850 beds and serves as a tertiary care centre and a teaching hospital.

STUDY DESIGN

This is a prospective study to demonstrate presence of Chikungunya antibody or viral particles in samples of patients clinically suspected for having dengue fever. Suspected dengue case is defined as an acute febrile illness characterized by frontal headache, retro-ocular pain, muscle and joint pain, and rash (WHO, 2006). A total of 49 blood samples were collected during study period.

SAMPLE COLLECTION AND PROCESSING

Adult patients (age >12 years old) attending Emergency Department of UKMMC with clinical features of dengue infection were included in the study. With the consent of the patient @5 ml blood was collected from each in a plain tube and transported in ice immediately to the Laboratory, Dept. of Microbiology and Immunology. All the samples were centrifuged at 3500 rpm for 5 minutes to obtain serum. The serum from each patient was divided into 4 tubes labeled 1 to 4 with specifically assigned laboratory number. The sample in the first tube was used to perform dengue serology. Only the samples with negative result for dengue serology: IgM and IgG were taken for further testing. The samples from tube number 2 and 3 were used to perform dengue and chikungunya RT-PCR, respectively. The tube numbers 4 of all were stored as backup samples. Results were tabulated and patients' clinical notes reviewed.

CHIKUNGUNYA SEROLOGY

Chikungunya Serology was performed using The Onsite® Chikungunya IgM Combo rapid test which employs lateral flow chromatographic immunoassay for qualitative detection of IgM-Chikungunya virus. The kit utilizes recombinant antigens derived from structural proteins. It detects IgM-anti-CHIK in patient serum or plasma within 15 minutes.

RNA EXTRACTION

RNA extraction from serum sample was performed using High Pure Viral Nucleic Acid Kit (Roche) according to manufacturer's instructions.

CHIKUNGUNYA REAL TIME REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION(RT-PCR)

The real-time PCR detection is based on the fluorogenic 5' nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

Chikungunya virus real time RT-PCR kit used in this study contains a specific ready-to-use system for the detection of the chikungunya virus by using Reverse Transcription Polymerase Chain Reaction in the real-time PCR system. The master contains a Super Mix for the specific amplification of chikungunya structural polyprotein gene which covers gene sequence for both the African and Asian lineage strains.

The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which the chikungunya virus RNA is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by means of polymerase chain reaction. Fluorescence is emitted and measured by the real time systems' optical unit during PCR. The detection of amplified chikungunya virus DNA fragment is performed in fluorimeter channel Cycling A.FAM of the PCR machine. External positive standards (CHIK S 1-5) contained 1×10^7 copies/ml supplied by manufacturer were used to allow determination of gene load.

In addition, the kit contains a system to identify possible PCR inhibition by measuring the VIC/JOE fluorescence of the internal control (IC). The PCR system used is Rotor Gene™ 3000 (Corbett Research Australia).

DENGUE REVERSE TRANSCRIPTION-PCR

PCR is used to amplify RNA. Using dengue-specific oligonucleotide primers, RT-PCR can detect a small number of dengue molecules. Initial step require viral RNA extraction, using High Pure Viral Nucleic Acid Kit (Roche). This is followed by amplification process similarly described

in Chikungunya PCR.

Statistical analysis: All statistical analyses were carried out using SPSS version 12.0.

RESULTS AND DISCUSSION

CLINICAL DIAGNOSIS

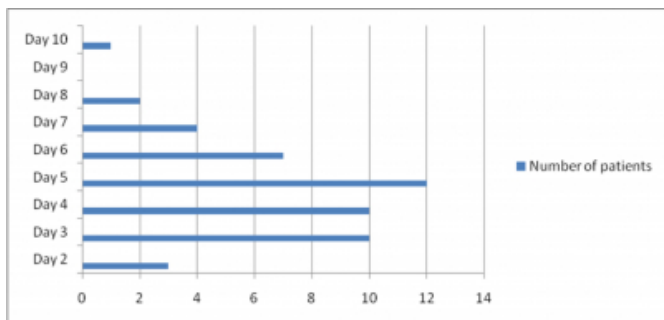
Blood samples obtained from 49 patients reported to emergency department University Kebangsaan Malaysia Medical Centre (UKMMC) those with clinically suspected dengue but serologically negative were included in the study. Clinical presentation was recorded from the patients at different stages those during included in the study.

Male-Female, Median age and Ethnic group: Fifty-seven percent of the patients were male with male- female ratio 1.32. The median age of the patients was 28 years (range 15 to 69 years). Distribution of ethnic groups were 62% Malays, 10% Chinese, 12 % Indian and 10% of other races.

Fever: Most of the patients presented fever during 1st 5 days of illness. The earliest presentation is on day 2 (3 patients or 6%) , 10 (20%) presented on day 3 and day 4 and 12 (24.4%) on day 5 (Fig.1).

Figure 1

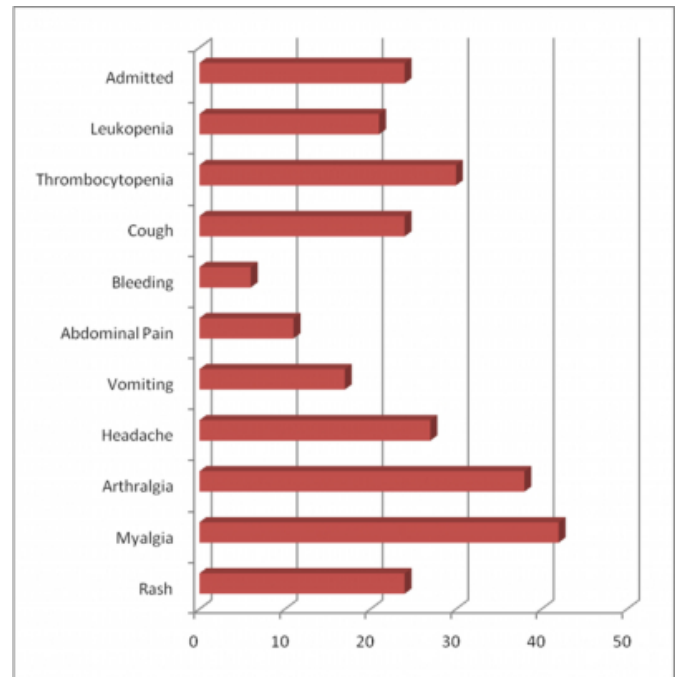
Fig.1 Days of fever of patients under study



Other common symptoms: All patients presented with fever. The other most common presenting clinical symptoms were myalgia (85%) and arthralgia (77%) (Fig. 2.).Rash were noted in 24 patients (49%), mainly involved the trunk and extremities. Other less common symptoms in this group of patients were abdominal pain (22%) and bleeding (12%).

Figure 2

Fig. 2.General clinical features of patients in the study



Serological diagnosis: Nineteen out of 49 patients (38.8%) were positive for chikungunya IgM . Others were proved to be negative for chikungunya by serology.

Real time polymerase chain reaction: All the patients sera were tested for chikungunya and dengue by PCR. It was observed that all the patients those serologically diagnosed as Chikungunya were found to be negative by PCR. On the other hand, nine patients showed positive for dengue by PCR.

Figure 3

Fig.3: Detection of dengue but negative for Chikungunya by RT- PCR

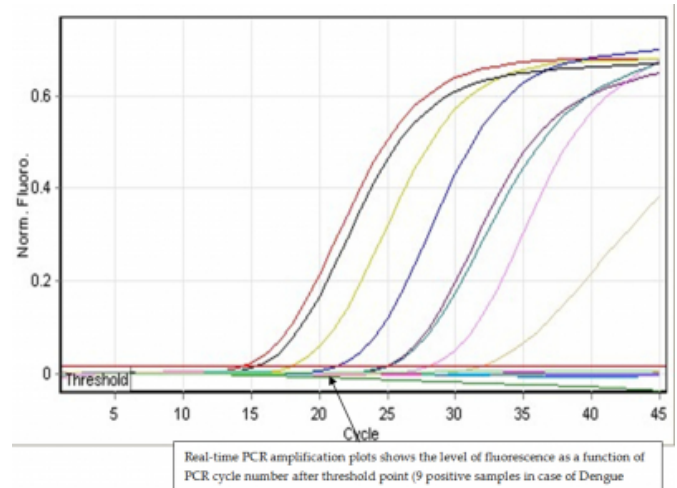


Figure 4

Fig. 4 :Standard curve for positive RT-PCR reactions

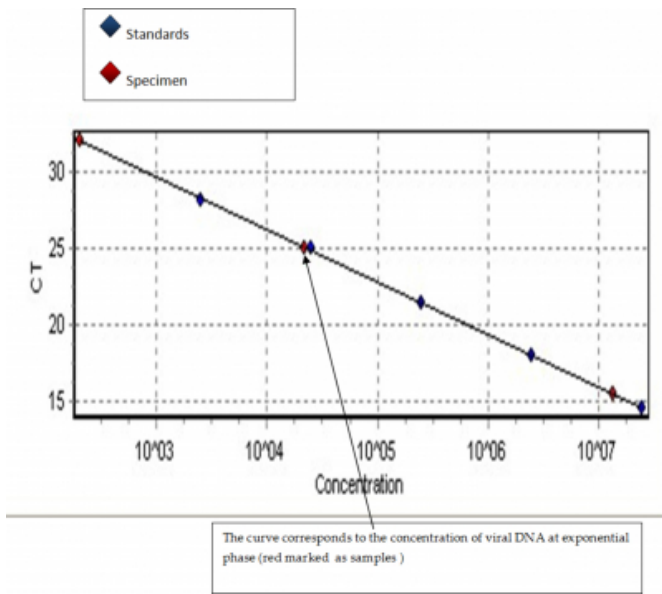


Fig.3. exhibits Real-time PCR amplification plots showing the level of fluorescence as a function of PCR cycle number after threshold point in 9 cases of dengue virus samples. Though with serology these samples were proved to be negative . Real time PCR result of chikungunya virus was not shown here as all the samples were found negative with this molecular study. Although 19 samples were proved to be positive by serology.

Fig.4 shows DNA concentration values obtained from software plotted in Microsoft Excel as a function of the concentration of cDNA. of dengue virus.

Figure 5

Fig. 5: Chikungunya and Dengue RT- PCR results

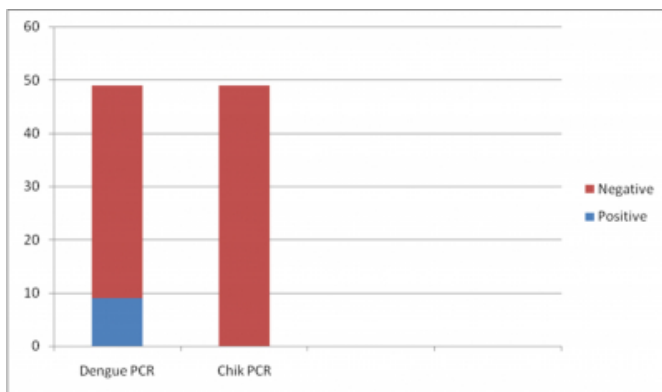
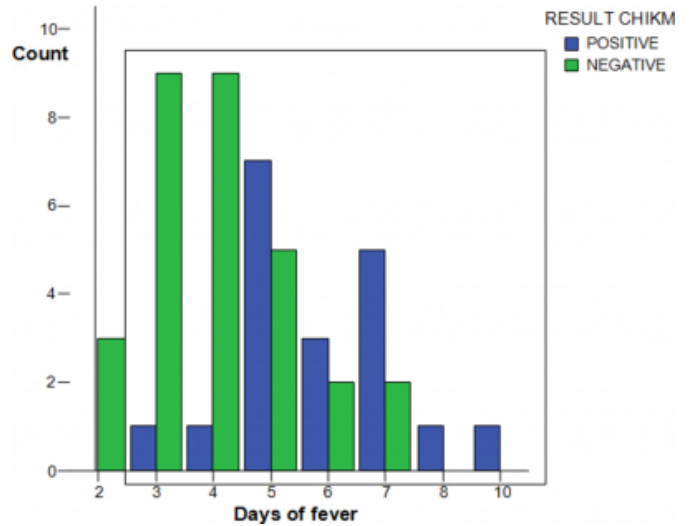


Fig.5. shows the comparative results of dengue and chikungunya as detected by RT-PCR . It reveals from the fig

that 9 patients sera those were serologically declared as negative for dengue were found to be positive by RT-PCR. On the other hand, all 49 sera samples were negative for chikungunya virus with this molecular method.

Figure 6

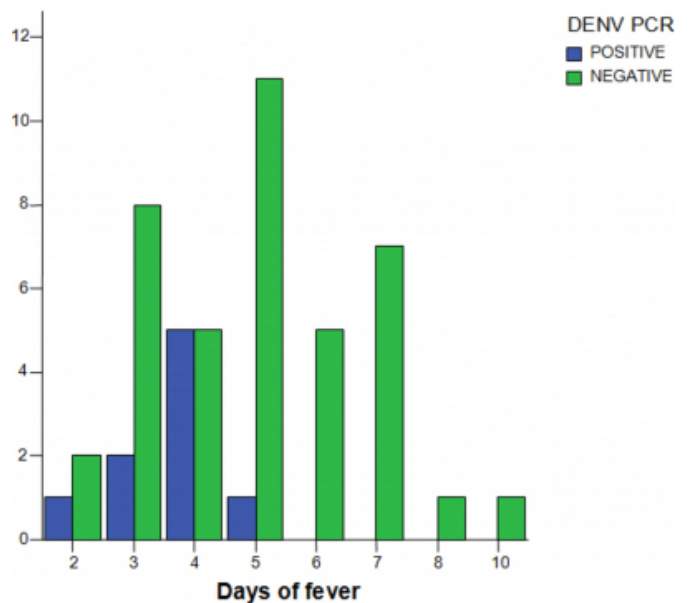
Fig.6:Day of fever of patients with Chikungunya IgM positive



Among patients with positive chikungunya IgM, 17 patients (89%) presented with 7 days of illness. Chikungunya IgM was detected as early as day 2 and up to day 10 of illness (Fig. 6)

Figure 7

Fig. 7:Day of fever of patients with Dengue PCR positive



All patients with positive detection of dengue PCR presented

with 5 days of illness. No patients were tested positive beyond day 5 (Fig.7)

Chikungunya virus (CHIKV) infection in Malaysia has gained attention since multiple outbreaks were reported by Lam et al. (2001) and AbuBakar et al. (2007) and last outbreak was recorded in 2008 (Barret, 2008). In the latest nationwide outbreak, our hospital was also involved in the care of the patients involved. Our aim in this study was to give importance of correct diagnosis with clinical features and confirmation of infection by laboratory methods. Although dengue fever that has been notoriously involved in Malaysian’s public health problems for decades also presents with similar clinical features, likelihood of chikungunya fever as a possible diagnosis should not be neglected.

This study looked into detection of chikungunya infection using serology and polymerase chain reaction. The study also included early clinical presentations of febrile patients with features of viral illness referred to Emergency Department (ED) at UKMMC. The patients with serologically confirmed dengue were excluded in the study. The patients constitutes of population enrolled at ED over a one year period from January 2009 and January 2010. Sampling was done randomly throughout the one-year during study period. The laboratory tests: serology and molecular method were performed to confirm the clinical diagnosis. Results have been described with figures and tables. Several other studies published and looked into early clinical and biological features of acute chikungunya infection. Simon et al. (2007) published a prospective observational study of clinical features of early chikungunya infections (within 10 days of disease onset). Taubitz et al. (2007) described clinical features and laboratory findings in 20 travellers on day 2 to 73 days after the onset of symptom. The results of the above study are in agreement of the present study. Clinical diagnosis of chikungunya based on clinical signs was examined by Staikowsky et al. (2009). They reported that the use of two cardinal signs in acute phase, fever and arthralgia have found a specificity of 99.6% and positive predictive value of 84.6%. However, as the clinical manifestations of chikungunya fever resembles to those of dengue and other fevers caused by arthropod-borne viruses of the genus alphavirus, laboratory confirmation is critical to establish the diagnosis (WHO, 2009). In our study it has been proved that laboratory study is prerequisite to declare finally patients infected with either chikungunya or dengue.

Figure 8

Table.1. Clinical features and statistical analysis of chikungunya diagnosed based on clinical basis and chikungunya IgM

	Chikungunya IgM POSITIVE n=19 (%)	p value	Chikungunya fever Clinical diagnosis n=7	p value
Rash	9 (47)	0.858	4 (57)	0.068
Myalgia	19 (100)	0.023*	7 (100)	0.708
Arthralgia	19	0.002*	7 (100)	0.160
Headache	10	0.782	4 (57)	0.322
Vomiting	3	0.027*	1 (14)	0.247
Abdominal Pain	2	0.111	1 (14)	0.062
Bleeding	1	0.235	0	0.110
Cough	7	0.176	1 (14)	0.404
Thrombocytopenia	10	0.326	5 (71)	0.684
Leukopenia	8	0.933	2 (28)	0.490
Admitted	6	0.052	2(28)	0.642

The classical clinical features of acute chikungunya infection are the triad of fever, arthralgia and inconstant skin rash (Robinson, 1955). Arthralgia is the key clinical features in clinical diagnosis of chikungunya infection. In this study, it is the main symptom experienced by patients with chikungunya IgM-positive group (p-value 0.002) and in CHIKV fever diagnosed clinically (p-value 0.160). Similar findings were reported by authors (Staikowsky et al., 2009; Win et al., 2010). Arthralgia in chikungunya is usually symmetrical and involved more than one joint. The pain can be excruciating and involved fingers, wrist, elbows, toes, ankles and knees. This type of arthralgia was described in most alphaviruses infection (Tesh, 1992). Robinson described the acute signs and symptoms were resolved after 2 weeks but arthralgia may persist for months or years. Borgherini et al. 2007 reported persistent and disabling arthralgia in more than 60 % of patients after 18 months. In a study of chikungunya virus infection it was observed that most working adults were disabled with loss of mobility, hand handicap, and depressive reaction which lasted for weeks to months. Clinical impact was much more severe in

older adults, progressively leading to complete loss of autonomy, health status deterioration, and sometimes death in debilitated or elderly people (Pialoux et al., 2007). Thus, by contrast with dengue fever, chikungunya virus fever is responsible for long-lasting consequences in health, social organization and economy in epidemic areas as reflected by the above findings.

Myalgia is also another consistent clinical feature seen in the 2 groups described above. It was present in all patients with clinical (p value= 0.708) and laboratory-confirmed chikungunya fever (p value 0.023). Findings in the clinically diagnosed chikungunya group were not statistically significant because of the small number (n=7). This clinical feature was also reported in other Malaysian study (Lam et al., 2001). Ng et al. (2009) conducted a comprehensive study on clinical and epidemiological features of chikungunya virus and observed more or less similar findings as we mentioned in clinical observations.

Rash is another common sign during acute illness. Win et al. (2010) and Queyriaux et al. (2008) reported rash was found in 54%-75% of patients infected with chikungunya virus. In our cohort, it was noted in almost half of the patient in CHIKV IgM-positive group (47 %). Smaller percentage was possibly because of difficulty in viewing erythematous macular rash in ethnic groups with darker skin colour (Lakshmi et al. 2008). Staikowsky et al. (2009) described the rash in chikungunya infection as morbiliform, roseola-like, maculopapular and few vesicular and bullous forms. In this study, most rashes were described as maculopapular, and no vesicular form was noted.

Atypical presentation of chikungunya were documented in several studies in large outbreak (Robin et al., (2008)). Atypical manifestations based on the systems affected such as neurological (encephalitis, seizures, neuropathy, ocular), cardiovascular (myocarditis, heart failure, arrhythmia), dermatological, renal (nephritis) and other miscellaneous manifestations were not documented in this study. The lack of atypical presentation in this group of patients is likely because high representation of younger age group (Pardigon 2008). Most patients also did not require admission likely due to mild degree of severity in their clinical presentation.

Fatality was not documented in this study. Earlier outbreaks in Reunion and India had few crude deaths reported (Mavalankar et al. 2007). In Malaysia the first case of mortality due to CHIK virus infection in Malaysia was reported in Sarawak in January 2010 (Chua et al. 2010). This

patient did not have pre-existing co-morbidity, developed hepatitis and succumbed likely due to cardiovascular collapse. Sam et al. (2010) later reported second death confirmed with viral culture in Kuala Lumpur in March 2010.

Although Ig M was known to last for months, using WHO criteria for diagnosis, we took the result of positive CHIK IgM as evidence of acute infection because all patients presented with fever (WHO 2006). Interestingly, among these 19 patients, only 6 was clinically diagnosed with chikungunya fever at presentation. These 6 patients gave history of traveling to other area affected with chikungunya fever and all has joint pain. The other 13 patients, 5 (26 %) were diagnosed as viral fever and nearly half, 8 (42 %) were diagnosed as chikungunya fever. It was noted that laboratory confirmation was essential for diagnosis of chikungunya fever.

Molecular tests such as the reverse transcriptase – polymerase chain reaction (RT-PCR) are useful for the diagnosis of dengue infection in the early phase (< 5 days of illness). It was shown to have a sensitivity of 100% in the first 5 days of disease, but reduced to about 70% by day 6, following the disappearance of the viraemia (Yong et al., 2007). In this study, similar findings were seen as dengue viraemia was detected in 9 cases (18.3%) of 49 patients who presented within 5 days of illness. This study did not look into serologically confirmed dengue because our aim was to detect chikungunya infection. Interestingly serologically positive chikungunya infection was not detected by RT-PCR. Regarding detection of chikungunya virus by molecular method Srikanth et.al(2010) mentioned that RT-PCR technique was useful in detecting the viral infection only in the acute phase and also indicated the usefulness of molecular techniques in an outbreak scenario. Our sera samples probably were not collected during acute phase of infection, for that negative results might have observed by RT-PCR.

This study was carried out in an area where dengue virus infection was endemic. Hence, co-infection with dengue virus infection was also very likely. Although patients with serology-positive dengue were excluded, co-infection if any could still be detected by PCR. However, no co-infection was found in the present study.

Biological similarities between dengue and chikungunya were observed. These arboviruses infections are associated with brief leucopenia (3 to 4 days) and thrombocytopenia. In

our cohort, group with CHIK IgM positive and dengue PCR show 52 % and 77 % respectively for thrombocytopenia. Leukopaenia was seen in 42% of CHIK IgM group and 55 % in dengue PCR positive group. These two biological parameters could be used to aid the diagnosis of chikungunya infection in acute phase in region free of dengue fever such as European countries (Staikowsky et al. 2009). However in dengue endemic region like Malaysia, these may not be as helpful as depicted in this study, higher proportioned was seen in dengue positive group.

In addition, WHO in its latest dengue guideline published in 2009, stated that new rational approach for management mainly involved early recognition and understanding of the clinical problems during the three different phases of the disease (febrile phase, critical phase and recovery phase). The patients are now classified into three classes; dengue with warning signs, dengue without warning signs and severe dengue. The most important laboratory investigations are serial haematocrit levels and full blood counts.

In conclusion, the present study highlighted important clinical observations of chikungunya and dengue viruses, their serological and molecular characteristics to rule out the present confusion and might be guidelines in early detection of the viral diseases so that appropriate management may be undertaken to the reduce long-lasting consequences in health.

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