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SERO-PREVALENCE OF CHIKUNGUNYA VIRUS AMONG ADULT POPULATION IN MANDALAY CITY, MYANMAR

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ABSTRACT

Context: Chikungunya, a mosquito-borne viral disease, was firstly identified in Tanzania in 1952 and then spread rapidly over 60 countries throughout Asia, Africa, Europe and the Americas since 2004. The global distribution of Chikungunya Virus (CHIKV) shows that this virus is expanding at an alarming rate. **Objective**: The study aimed to find out the sero-prevalence of CHIKV and associated factors among adult population. **Methods**: This cross-sectional study was conducted from July to October 2020 among 90 respondents aged over 18 years, who were randomly selected from 6 townships of Mandalay city, Myanmar. **Results:** Seropositivity rate of CHIKV was 74.44% and those of IgM and IgG were 6.67% and 74.44%, respectively. Seropositivity of female gender (80.36%),high education (76.92%), daily wage worker (88.89%), many family members (84.13%), and lower family income (75.71%), living in a house without mosquito screen (81.08%) were higher than their counterparts. The participants with self-reported history of CHIKV infection, and history of joint pain also had higher seroprevalence (93.33% and 88.24%) than their analogue. Seropositivity was significant for high education (AOR 7.63; 95%CI 3.24–17.02), having ≥4 family members (AOR 3.68, 95%CI 2.97-14.03), being house without mosquito screen (AOR 16.73, 95% CI 2.27-23.14), and those with history of CHIK V infection (AOR 10.89, 95%CI 1.33-19.12) with p <0.05. **Conclusion:** Seroprevalence survey should be conducted on a regular intermittent premise particularly in individual at risk. People should be encouraged to be aware about the silent infection of CHIKV infection.

KEYWORDS: Sero-prevalence, Chikungunya, adult, Myanmar.

INTRODUCTION

Chikungunya fever (CHIKF) has re-emerged as an important mosquito-borne disease caused by the Chikungunya virus (CHIKV), an *Alphavirus* belonging to the *Togaviridae* family[1], and transmitted by two main vectors, *Aedes aegypti* and *Aedes albopictus* in the urban cycle.[2] It is characterized by fever, joint pain, headache and myalgia.[3] High fever, headache, myalgia, arthralgia, polyarthralgia, hemorrhage, and rash are the typical clinical signs of CHIKV fever.

The disease was first described during an outbreak in southern Tanzania in 1952.[4] Since then, CHIKV outbreaks had been identified in countries in Africa, Asia, Europe, and the Indian and Pacific Oceans.[5] The global distribution of CHIKV shows that this virus is expanding at an alarming rate. This global health menace has affected millions of people in tropical and sub-tropical regions of the world. In Asia, CHIKV was first isolated in Bangkok, Thailand, in 1958, and outbreaks of CHIKF have been reported since the 1960s.[6,7] More recent reports of CHIKF outbreaks in Southeast Asia include those of Indonesia in 2001- 2003[8], Malaysia in 2006-2009[9,10], Thailand in 2008-2009[11] and Singapore in 2008-2009.[12]

The diagnosis of chikungunya has been challenging due to the similarity of the clinical symptoms to those of dengue. In order to overcome this challenge, the researchers have used multiplex real-time RT-PCR assays that quantitate and detect RNA for all CHIKV serotypes and dengue virus serotypes.[13]

The dramatic spread of CHIKV in recent years highlights the urgent need to take precautionary measures, as well as to investigate options for control. The current status of research with regard to CHIKV is not encouraging because there is no proper serological test, vaccine, treatment, or vector control program. Public health officials and medical professionals need to work on critical areas of research to overcome these challenges so

that an explosive increase in CHIKV cases can be mitigated.[14]

Chikungunya infection is diagnosed on the basis of clinical, epidemiological and laboratory criteria. An acute onset of fever and severe arthralgia or arthritis that is not explained by other medical disorders is considered a possible CHIKV case.[15] Three main types of laboratory tests are used for diagnosing CHIKV infection: virus isolation, reverse transcriptase-polymerase chain reaction (RT-PCR), and serology. Virus isolation can be performed on field collected mosquitoes or acute serum specimens (≤8 days). Serum obtained from whole blood collected during the first week of illness can be inoculated into a susceptible cell line or suckling mouse at a reference laboratory. This can be achieved if the sample is transported cold (between 2◦C and 8◦C or dry ice) and as soon as possible (within 48 h).[16]

Chikungunya virus can be diagnosed by viral culture but the facilities needed for culturing the viruses are not widely available in many underdeveloped and developing countries. Molecular tools such as RT-PCR and sero-diagnostic methods like Enzyme-linked immune-sorbent assay, indirect immune-fluorescent method, hem-agglutination inhibition, or neutralization techniques for the detection of IgM and IgG antibodies against Chikungunya virus in sera can also be used.[2][16]

Persistence of IgM antibodies was found to be varying in different studies and it did not persist normally more than three-four months period, at detectable levels.[17] However, IgG antibodies are reported to be detectable in convalescence and remains so for years.[17]

Mandalay is one of the regions of Myanmar where epidemics of Chikungunya was reported. There was probable outbreak of suspected cases of Chikungunya in Mandalay city within previous one to two years. Clinical cases of people with high fever, headache, myalgia, arthralgia, polyarthralgia, hemorrhage, and rash and even chronic joint pains were recorded among general practitioners and clinicians in different hospitals in Mandalay during previous one to two years. However, there has been limited information on the seroepidemiology of CHIKV in Mandalay, Myanmar. This study aims to assess the sero-prevalence of CHIKV and its associated factors among adults residing in selected townships of Mandalay city.

MATERIALS AND METHODS

A cross-sectional study was conducted from July to October 2020 among 90 respondents aged over 18 years, living in 6 townships of Mandalay City, Myanmar. Mandalay city is a third capital of Myanmar and it is in central Myanmar. There is total 6 townships in the city. One ward from each township was randomly chosen and then 15 households from each ward was randomly selected. One adult from randomly selected households were recruited. The study aimed to find out the sero-prevalence of CHIKV and its associated factors among adults residing in Mandalay city during 2020. Structured questionnaire was used enquiring for socio-demographic characteristics and clinical history of chikungunya fever.

Serum collection

Three milliliter of intravenous blood was collected into the commercially available coded bottles not containing anti- coagulants by venipuncture and the samples were sent to microbiology department of University of Medicine, Mandalay on the same day. At Laboratory, the samples were leaved to settle for 30 minutes for blood coagulation and then centrifuged blood to get serum specimen of supernatant. Serum in the plain tube was stored in a refrigerator at 4°C until testing.

Chikungunya Antibody Detection

The samples were detected for seropositivity of CHIK V IgG, by using the commercially available anti-Chikungunya IgG IIFT EUROIMMUN test kits (ERUOIMMUN AG, Medizinische Labordiagnostika AG, Luebeck, Germany). Kit components and collected sample were allowed to room temperature (30°C) a minimum of 30 minutes prior to testing.

A STANDARD F100 Analyzer was prepared and was set the Standard Test mode according to the analyzer's manual. The test device was inserted to the Test Slot of the analyzer. The analyzer was automatically read the information of the bar code on the test device and released the test device for adding sample. The 10µl of serum was collected to the black line of a STANDARD Ezi tube. The collected serum was added to the sample well of the test device. Three drops of assay diluent were added into the assay diluent well of the test device. After applying the sample and buffer, the center button was immediately pressed to start the test. When human anti-Chikungunya IgM or IgG existed in patient serum, complexes with anti-human IgM/IgG on the test lines, human IgM/IgG in patient sample, inactivated Chikungunya virus, and anti-ChikungunyaEnv-Ep made fluorescence signal. The intensity of the fluorescence light generated on the membrane was scanned by the STANDARD F Analyzer manufactured by SD BIOSENSOR. STANDARD F Analyzer analyzed the presence of the analyte in the specimen by processing the results using pre-programmed algorithms and displayed the test result on the screen. The analyzer automatically displayed the test result after 15 minutes. STANDARD F Analyzer read the fluorescence signal of the internal procedural control zone and decided whether the result is valid or invalid for quality control.

Data Processing and Data Analysis

Categorical variables were presented by frequency distribution tables. Association between seropositivity, the sociodemographic characteristics, and clinical history of CHIK F were analyzed by Chi Square test or Fisher Exact test whatever appropriate. Multiple logistic

regression analyses were conducted to explore the factors associated with seropositivity of CHIK V infection. Unadjusted odd ratios (UORs), adjusted odd ratios (AORs), and 95% confidence intervals (95% CI) were calculated, setting P-value <0·05. IBM Statistical Package for the Social Sciences (SPSS) version 22.0 was used for data analyses.

Ethical Consideration

This research was approved by Institutional Review Board of University of Medicine, Mandalay with the number (ID No 409(REC)/UMM/2020). Informed consent was obtained from selected participants after thorough explanation of the purpose, procedure, and possible consequences of the research. Confidentiality of the information collected was strictly maintained. Institutional standard guidelines were followed for the collection of blood samples after obtaining their written informed consent for participation in the study. The results of this study were used for the health care and research purpose only.

RESULTS

Among the 90 serum specimens tested, IgM or IgG antibodies to CHIKV were detected in 67 (74.44%). IgM antibodies were detected only in 6 (6.67%) of the sera and IgG antibodies were found in 67 (74.44%). Almost all seropositive sera which had IgM antibodies were also positive in IgG. (Table 1)

Table 1: Seropositivity of Chikungunya virus infection in Mandalay City, Myanmar (N=90).

	IgG	IgM	IgG &or IgM
Positive	67 (74.44)	6(6.67)	67 (74.44)
Negative	23(25.56)	84(93.33)	23(25.56)
Total	90(100)	90(100)	90(100)

Table 2 reveals the seropositivity of CHIK V among the study population characteristics. CHIK V seroprevalence was high in working age group (~70%) suggesting that working individuals in Mandalay city have had experienced a hazard of infection. Seropositivity among the elderly population was vitally high (90%). Seropositivity of female gender (80.36%), low education level (76.92%), daily wage worker (88.89%), having four and more family members (84.13%), and earning lower monthly family income (75.71%), living in a house with mosquito screen (81.08%) were higher than their counterparts. The study participants with self-reported history of CHIK V infection, those with treatment taken history and history of joint pain also had higher seroprevalence (93.33%, 90% and 88.24%) than their analogue.

Table 2: Socio-demographic characteristics of the study population by seropositivity.

Variables		Seropositivity (%)	SE	95% CI	
Age Group (years)	<20	85.71	14.29	37.14	98.39
	20-39	70.27	7.62	53.39	82.99
	40-59	72.22	7.57	55.12	84.62
	≥60	90	10	49.74	98.79
Sex	Male	64.71	8.32	47.06	79.08
	Female	80.36	5.36	67.58	88.93
Education	Low	73.44	5.56	61.07	82.97
	High	76.92	8.43	56.48	89.54
Occupation	Employee	75	9.03	53.54	88.65
	Dependent	75	9.93	51.15	89.58
	Daily wage worker	88.89	7.62	63.32	97.37
	Business owner	64.29	9.22	44.76	79.99
Family members	<4	51.85	9.8	33.05	70.14
	≥4	84.13	4.64	72.65	91.36
Income (MMK)	≤300,000	75.71	5.16	64.09	84.49
	>300,000	68.42	10.96	44.16	85.58
House	With mosquito screen	43.75	12.81	21.66	68.63
	Without mosquito screen	81.08	4.58	70.3	88.59
Daytime sleeping habit	Never sleep	69.23	13.32	39.37	88.63
	Ever sleep	75.32	4.95	64.28	83.82
History of CHIK V *	Yes	93.33	4.63	76.13	98.4
	No	65	6.21	76.13	98.4
Treatment taken	Yes	90	6.88	65.32	97.73
	No	80	13.33	42.11	95.65
History of joint pain	Yes	88.24	8.05	60.64	97.33
	No	80	10.69	50.59	93.99

** Self-reported*

Table 3 shows the multiple logistic regression of seropositivity of CHIK V infection by sociodemographic characteristics. Age over 60 years had 52% higher chance of getting seropositivity than under 20 years. Although being female gender had 2.45 odds higher risk, daily wage worker occupation had 4 odds higher chance, and having income more than 300,000 (MMK) had 2.19 odds higher chance to be seropositive than the counterpart references, there was no statistically significant association. The adjusted odds ratio of having seropositive was significant for having high education level (AOR 7.63; 95%CI 3.24–17.02), having ≥4 family members (AOR 3.68, 95%CI 2.97-14.03), being house without mosquito screen (AOR 16.73, 95% 2.27-23.14), and those with history of CHIK V infection (AOR 10.89, 95% 1.33-19.12) with p <0.05.

Table 3: Multiple logistic regression of seropositivity of Chikungunya infection by sociodemographic characteristics (N=90).

Variables		UOR	AOR	95% CI
Age Group (years)	<20	Ref	Ref	
	20-39	0.39	0.19	0.00 - 33.83
	40-59	0.43	0.39	0.00 - 64.70
	≥60	1.5	1.52	0.01 - 34.54
Sex	Male	Ref	Ref	
	Female	2.23	2.45	0.65 - 9.14
Education	Low	Ref	Ref	
	High	1.21	7.63**	3.24 - 17.02
Occupation	Employee	Ref	Ref	
	Dependent	1	0.51	0.06 - 4.20
	Daily wage worker	2.67	4.09	0.37 - 15.05
	Business owner	0.6	0.67	0.14 - 3.26
Family members	<4	Ref	Ref	
	≥4	4.9	3.68*	2.97 - 14.03
Income (MMK)	≤300,000	Ref	Ref	
	>300,000	0.69	2.19	0.43 - 11.20
House	With mosquito screen	Ref	Ref	
	Without mosquito screen	5.51	16.73***	2.27 - 23.14
Daytime sleeping habit	Never sleep	Ref	Ref	
	Ever sleep	1.15	0.76	0.17 - 3.50
History of CHIK V *	No	Ref	Ref	
	Yes	7.53	10.89**	1.33 - 19.12
Constant			0.005	0.00 - 0.60

**** p<0.01, ** p<0.05*

** self-reported*

DISCUSSIONS

High seropositivity (81%) among living in the house without mosquito screen and those with day time sleeping habit was because of As Mandalay is a tropical region with high density of mosquitoes, houses without mosquito screen are very risky to get mosquito bites. As Chikungunya virus is transmitted through the bite of Aedes mosquito which is a day bite vector, day time sleeping habit is very risky to get Chikungunya virus infection, unless mosquito nets are used properly.

Mindfulness of the extent of CHIKV endemicity in Myanmar is imperative in assisting the determination and control of mosquito-borne viral infection. In spite of the fact that they account for significant horribleness in Myanmar, the degree of the viral burden is regularly clouded by inadequate surveillance and clinical misdiagnosis. The overall prevalence of CHIK V infection in our participants was 74.44% which was within the range of prevalence between 0% and 76% globally[19] but it was significantly lower than overall CHIKV seropositivity in Thailand, a study conducted by Vongpunsawad et al, 2017.[20] Seropositivity of IgM and IgG in this study were 6.67% and 74.44% which was the opposite findings to those in the study done in Rande Comore Island, Union of The Comoros, 2005 whereas IgM was 60% and IgG was 27%.[21] Also seropositivity of IgG was higher than that in a study conducted in Kerala, India, 2007.[22]

In fact, comparisons of seroprevalence among studies totally different nations are complicated by the contrasts within the socioeconomics of the study population, stratification of age groups, viral antibody discovery utilized, and research method. Seropositivity was higher in female gender (80.36%) which was contradicted the findings in Singapore resident population whereas men had significantly higher CHIKV seroprevalence than women.[23] But Vongpunsawad et al, 2017 mentioned that CHIKV seroprevalence was no significant

differences among gender (26.1% male versus 27.5% female).[20] Though the observations that elderly people with positive CHIKV (90%) was also higher than that of Singaporean population , age ≥58 years as the protective factors the study of Chongsuvivatwong and Thammapalo conducted in the southern Thailand, 2013.[24] The individuals younger than twenty years were more likely to be CHIKV seropositive compared to working populations and similar pattern was seen in Thavara et al, study conducted in central Thailand, 2009.[25]

Having a high level of formal education as a safeguarding factor in that Thailand study is also contradicted with this study where higher prevalence in higher education group.[25-27]

Seropositivity of CHIK V among those with self-reported history of CHIK fever symptoms within one year was high showing the clinicians' report of high incident and prevalence of CHIK V fever symptom in Mandalay during a couple of years ago is reliable.

About 65% of persons who did not have history of CHIK fever symptoms within one year is sero-positive of CHIK V. It may be due to silent infection or because of only one year period to take CHIK fever history. If the silent infection rate is high, it may be difficult to prevent transmission of the disease. However, IgG antibodies are reported to be detectable in convalescence and remains so for years.[28] Therefore, CHIK V IgG detected in this study may be the residual of infections beyond previous one year.

CONCLUSION

Female population, the elderly and daily wage workers are more vulnerable to CHIK V infection. There is significant association between number of family members, house having mosquito screen, having history of CHIK fever symptoms within one year and sero-positivity of CHIK V IgG. Seroprevalence survey should be conducted on a regular intermittent premise particularly in individual at risk. People should be encouraged to be aware about the silent infection of CHIK V infection, which is dangerous source of infection, by effective health education policies. As IgG antibodies are reported to be detectable in convalescence and remains so for years, seroprevalence studies alone are not enough evidence to show incident rate of that infection.

Therefore, research to detect actual incident rates should be performed in this area.

LIMITATION OF THE STUDY

This study can give only the seroprevalence of IgG which can be found in the sera within a year after the attack of CHIK V infection. Currently infected patients could not be distinguished by only IgG. The study samples were limited because of the semi lockdown of the city during the study period due to COVID-19 infection.

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DECLARATIONS

Conflict Of Interest

The authors have declared that there was no conflict of interests.

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AUTHOR CONTRIBUTIONS

Conceptualization, PPS, YYW, and KTDS; methodology, PPS, YYW, KTDS and WMO; formal analysis, PPS and YYW; investigation, all authors; writing—review and editing, YYW and PPS; supervision, WMO; project administration, PPS and WMO; funding acquisition, YYW. All authors have read and agreed to the published version of the manuscript.

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