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Seroprevalence and clinical manifestations of Chikungunya infection in Makassar city, Indonesia

Seroprevalencia y manifestaciones clínicas de la infección por Chikungunya en la ciudad de Makassar, Indonesia

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SUMMARY

Background: *Chikungunya is a vector-borne disease with a high morbidity rate, spread in dengue hemorrhagic fever endemic areas because the vector carrying the virus is transmitted by the same mosquitoes, namely Aedes Aegypti and Aedes Albopictus. The number of mosquito breeding sites is often associated with an increased incidence of chikungunya disease. The proximity of mosquito breeding sites to human habitation is a significant risk factor for chikungunya. Data related to chikungunya is still lacking in Makassar. This study aims to understand the prevalence of CHIKV infection through serodiagnosis by measuring CHIKV IgG antibodies and looking at the history of clinical symptoms associated with CHIKV infection in Makassar to reduce widespread transmission.*
Methods: *This study used a cross-sectional study design in 5 DHF-endemic areas in Makassar City. Blood samples and questionnaires were collected from 102 respondents from August to September 2023. ELISA technique was used to examine anti-CHIKV IgG. Data were analyzed using a Student t-test. Variables with a probability value (P-value) of less than 0.05 were considered statistically significant. The processed data was presented as frequency distribution tables and bar charts to see the relationship between independent and dependent variables.*
Results: *29.4 % of respondents were identified to be exposed to CHKV (IgG positive). The main clinical symptoms were fever and arthralgia.*
Conclusion: *The findings of this study provide evidence of CHIKV exposure in Makassar city.*

Keywords: *Chikungunya, Seroprevalence, Clinical Manifestations, ELISA.*

RESUMEN

Antecedentes: *El chikungunya es una enfermedad transmitida por vectores con una alta tasa de morbilidad, que se propaga en áreas endémicas de fiebre hemorrágica del dengue porque el vector que lleva el virus es transmitido por los mismos*

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mosquitos, a saber, Aedes Aegypti y Aedes Albopictus. El número de criaderos de mosquitos a menudo se asocia con un aumento en la incidencia de la enfermedad de chikungunya y la proximidad de los criaderos de mosquitos a la vivienda humana es un factor de riesgo significativo para el chikungunya Los datos relacionados con el chikungunya aún son escasos en Makassar. Este estudio tiene como objetivo comprender la prevalencia de la infección por CHIKV a través del serodiagnóstico mediante la medición de anticuerpos IgG contra el CHIKV y observar la historia de síntomas clínicos asociados con la infección por CHIKV en Makassar, con el fin de reducir la transmisión generalizada.

Métodos: *Este estudio utilizó un diseño de estudio transversal en cinco áreas endémicas de fiebre hemorrágica del dengue en la ciudad de Makassar. Se recopilaron muestras de sangre y cuestionarios de 102 participantes durante agosto a septiembre de 2023. Se utilizó la técnica de ELISA para examinar los anticuerpos Anti-CHIKV IgG. Los datos se analizaron mediante la prueba de t de Student. Las variables con un valor de probabilidad (valor p) menor a 0.05 se consideraron estadísticamente significativas. La presentación de los datos procesados se realizó en forma de tablas de distribución de frecuencias y mediante gráficos de barras para observar la relación entre las variables independientes y dependientes.*

Resultados: *Se identificó que el 29,4 % de los participantes estuvieron expuestos a CHKV (IgG positivo). Los principales síntomas clínicos fueron fiebre y artralgia.*

Conclusión: *Los hallazgos de este estudio proporcionan evidencia de la exposición al CHIKV en la ciudad de Makassar.*

Palabras clave: *Chikungunya, seroprevalencia, manifestaciones clínicas, ELISA.*

INTRODUCTION

Chikungunya (CHIKV) is a vector-borne disease with high morbidity and substantial health and socioeconomic impacts. Still, it is often overlooked as an important cause of fever in tropical and subtropical regions (1). Human CHIKV infection is generally described as a self-limiting febrile illness with sudden onset, usually accompanied by headache, myalgia, rash, and characteristic arthralgia. An association of CHIKV infection with neurological complications has also been reported, but to date, remains poorly documented (2).

Makassar City is one of the dengue endemic areas with an incidence rate for 2021 of 39.3 % per 100 000 population and a case fatality rate (CFR) of 0.2% (3). The spread of chikungunya disease in Indonesia occurs in dengue hemorrhagic fever endemic areas because the vector carrying the virus is transmitted by the same mosquitoes, *Aedes aegypti* and *Aedes albopictus*. An observational study conducted by the *Indonesia Research Partnership on Infectious Diseases* (INA-RESPOND) in 8 hospitals across Indonesia from 2013 to 2016 reported acute CHIKV infection (ACI) was most prevalent in Makassar (7 %), followed by Semarang (5.2 %). Previous CHIKV exposure, determined by IgG serology, was also highest in these two cities (37.9 % and 45.9 %, respectively) (4).

Unlike Dengue Fever (DHF), which is widely recognized, underdiagnosis of chikungunya is still common in Indonesia. This is because the clinical presentation/symptoms of chikungunya are challenging to distinguish/overlap with dengue fever and other endemic disease infections and the lack of diagnostic testing capacity. Symptomatic surveillance alone may not be a reliable indicator of infection for many tropical diseases, but serological confirmation effectively estimates pathogen exposure in a population (5). Many areas in Indonesia do not have a specific diagnostic test for chikungunya. In the clinical setting in Indonesia, Dengue Virus (DENV) infection should be considered first as the primary diagnosis to minimize the incidence of severe forms of dengue fever and, therefore, to reduce mortality from dengue. This rationale is based on the fact that dengue fever more often presents in severe forms compared to CHIKV and has a high mortality rate in Indonesia (6).

The lack of seroepidemiological data in Makassar suggests significant knowledge gaps regarding the burden and susceptibility of chikungunya disease in dengue-endemic areas. However, pre-existing immunity to CHIKV does not appear to exacerbate reinfection by other CHIKV progeny. Therefore, periodic seroprevalence surveys are important to evaluate the immunity of a population and appropriate public health interventions such as vaccination. Here, we assessed past exposure to CHIKV by

evaluating pre-existing IgG antibodies to this virus.

Available data on the prevalence of Chikungunya in Indonesia, especially in Makassar, is still lacking. Therefore, this study was conducted to understand the prevalence of CHIKV infection through serodiagnosis by measuring CHIKV IgG antibodies to reduce widespread transmission.

MATERIALS AND METHODS

Study Design and Participants

This cross-sectional study was conducted between July and August 2023 in Makassar City. The region was selected by considering the prevalence of DHF because the same mosquito transmits Chikungunya and DHF infections. The selected areas were Kaluku Bodoa, Jumpandang Baru, Pattingaloang, Tamamaung, and Bangkala, dengue-endemic areas. The population in this study was the community in these five areas. Samples were selected using a simple random sampling design. A total of 102 participants were selected. Those who were willing to participate in the study were those who signed the consent form.

Instruments and Procedures

Home visits carried out interview procedures and blood sampling on respondents. A verbal explanation of the study's purpose, benefits, and procedures was given to each respondent, and only respondents who were willing to sign the consent form were included as research participants. Each respondent was asked about gender, age, education, occupation, and history of clinical symptoms that had been experienced.

Blood draw and Laboratory Procedures

Three milliliters of blood per venous puncture from each participant were drawn using a vacuum tube. The blood was centrifuged to separate the cells from the serum, and the serum was stored at 2-8°C if the assay was performed within five days after sampling; otherwise, the sample was separated and frozen (-20 to -80°C).

IgG antibody levels for CHIKV were measured using a kit from ABCAM with lot number GR3440497-1. This kit provides an Anti-human (IgG) coated microplate (12x8 wells), IgG sample diluent, stop solution, 20x washing solution, chikungunya virus antigen solution 1, chikungunya virus biotinylated antibody Solution 2, streptavidin conjugate, 3,3',5,5'-Tetramethylbenzidine Liquid (TMB) Substrate System for ELISA, Positive control, negative control, cut-off control. The IgG ELISA measurement process was carried out following the manufacturer's procedures.

Data were interpreted as follows: <9 negatives, indicating no previous CHIKV infection; Events 9-11 were considered equivocal (a grey area); and >11 positives, indicating previous CHIKV infection. No retesting was performed for equivocal results, and they were considered negative.

Data Analysis

Data were analyzed using the SPSS program with the Studentnt-test. Variables with a probability value (P-value) of less than 0.05 were considered statistically significant. The data was presented in frequency distribution tables and bar charts to establish the relationship between independent and dependent variables.

Ethics Approval

This study was approved by the Research Ethics Committee of the Faculty of Medicine, Hasanuddin University Makassar, with ethical approval recommendation number 351/UN4.6.4.5.31/PP36/2023. Written informed consent was sought and obtained from all adults (>18 years) and consent from parents/guardians of children (<18 years) before recruitment into the study. Records of all participants were documented through identification numbers for anonymity.

RESULTS

A total of 102 participants were involved in this study. The group comprised 35 male respondents (34.4 %) and 67 female respondents (65.7 %). Their ages ranged from 11 to 75 years old, with a mean of 34.9; most were 20-59 years old (64.7 %). Positive CHIKV IgG antibodies were detected in 30 respondents (29.4 %). CHIKV infected more female groups, namely 23 (76.6 %) people. CHIKV infection was found in all age groups. Still, it was more common in respondents of productive / adult age (20-59 years), with a moderate level of education (junior / senior high school) at 63 %, primarily working as housewives 17 (56.67 %) people (Table 1). There is no significant relationship between demographic characteristics and chikungunya seropositivity.

Table 1. Demographic characteristics of respondents

Respondent Characteristics	n	IgG (%)	P-value
Overall	102	30 (29.4)	
Sex			
Male	35	7 (23.3)	0.132
Female	67	23 (76.6)	
Age (year)			
<20	25	3 (10,0)	0.063
20-59	69	25 (83.3)	
60+	8	2 (6.7)	
Education level			
TS-SD	30	6 (20,0)	0.387
Junior/Senior High School	61	19 (63.3)	
University	11	5 (16.7)	
Occupation			
Students	23	3 (10,0)	0.149
House Wife	54	17 (56,7)	
Government Employ	8	4 (13.3)	
Others	17	6 (20.0)	

The prevalence of CHIKV IgG in five areas in Makassar varied between 3.33 % to 43.33 %. This study found the highest prevalence of positive CHIKV IgG antibodies in the Pattingaloang area at 43.33 %. The number of respondents participating in each area varies (4-30), but the percentage (3.3-43.3 %) of respondents who are positive for CHIKV IgG antibodies shows that none of the areas are free of chikungunya virus (Table 2).

Table 2. Distribution of CHIKV in DHF Endemic Areas in Makassar City

Sub District	Number of Samples	Number of positive	% of positive cases
Bangkala	4	4	13.33%
Tamamaung	19	1	3.33%
Jumpandang Baru	19	4	13.33%
Kaluku Bodoa	30	8	26.67%
Pattingaloang	30	13	43.33%

Data regarding the history of clinical symptoms that had been experienced were also asked of respondents (Figure 1). Typical clinical manifestations such as fever, arthralgia, myalgia, headache, and petechias were found in respondents with positive IgG. Based on the results of the Student t-test (Table 3), the average ELISA value for those with a history of fever was 12.86, petechias 14.67, headache 10.25, myalgia 10.86, and arthralgia 13.39. With a p-value of fever (0.0001), arthralgia (0.0001), and petechias (0.015), this shows that these three symptoms are significant in respondents who are positive for chikungunya IgG.

Table 3. History of clinical symptoms

Clinical symptoms		ELISA values		Independent T-test	
		Mean	SD	t	p
Fever	with	12.86	9.18	4.94	0.0001
	without	5.63	3.89		
Petechiae	with	14.67	8.77	2.12	0.015
	without	8.26	7.31		
Headache	with	10.25	8.52	1.077	0.28
	without	8.36	7.31		
Myalgia	with	10.86	9.25	1.93	0.56
	without	7.81	6.51		
Arthralgia	with	13.39	8.70	4.805	0.0001
	without	5.99	5.20		

The main symptoms of chikungunya are fever and joint pain. Other common signs and symptoms include muscle pain, rash, and headache.

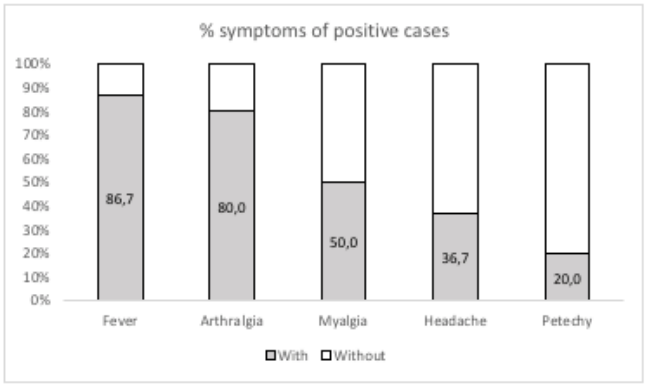

Figure 1. History of clinical symptoms in respondents with positive IgG.

DISCUSSION

This study showed a chikungunya seroprevalence of 29.4 %, which was detected by the presence of anti-CHIKV IgG in the serum of respondents. This is not much different from the study of Vongpunsawad et al. in Thailand, who reported that the research subjects had scheduled health checks or outpatients at 26.8 % (7). However, in the same area, this seroprevalence rate is lower than the seroprevalence reported by

Jalloh et al. on blood donor samples collected from the Makassar Health Laboratory Centre (BBLK), which was 53.9 % (8). In contrast, research conducted by Azami et al. in Malaysia showed a lower seroprevalence of only 5.9 % of the healthy adult population in non-outbreak areas (9). The difference in seropositive in this study and other studies may be due to differences in demographics, study period, sample size, and methodology.

Seroprevalence in this study was higher in the female group and at productive age (20-50 years). This result is in line with the study of Pratamawati et al., which also showed that women were more affected by chikungunya (10). However, other reports indicate that men are more susceptible to CHIKV infection than women (11). The difference in the results of this study is related to vector activity and the activity of the sufferer. In this study, the most common occupation of the participants was housewife, which indicates that the potential for transmission is possible around the house where individuals stay at home during chikungunya vector biting hours. The biting activity of the chikungunya vector *Aedes sp* usually starts in the morning and evening. *Ae aegypti* and *Ae. albopictus* are the only mosquitoes believed to transmit CHIKV regularly in settings involving human amplicides (12). However, these results should be interpreted cautiously as more females were in this study.

The areas in this study belong to densely populated urban areas, and there were anti-CHIKV IgG-positive samples from all regions. This may increase the risk of exposure to disease vectors. In particular, *Ae aegypti*, an anthropophilic mosquito species known to transmit the virus, thrives near human habitation, exacerbating the risk of transmission in densely populated areas. The urban-dwelling mosquito species *Ae. aegypti* plays an important role in the transmission of Dengue Virus (DENV), Zika Virus (ZIKV) and Chikungunya Virus (CHIKV) worldwide. These mosquitoes thrive in densely populated urban areas and their surroundings, largely due to their preference for a diet of human blood and the presence of man-made breeding sites, such as water containers and old tyres (13). The transmission pattern of the virus is influenced by various factors, including the virus itself, the mosquito and its human host, and environmental conditions. In addition, the coexistence of multiple arboviruses may encourage new interactions among arboviruses, making transmission dynamics more complicated (14). Surveys of Aedes larvae and mosquitoes conducted in the Kaluku Bodoa area of Makassar City, specifically in Ujung Pandang Baru and Pannampu villages, showed moderate (<37 %) and high (>50 %) HI values, respectively (15).

Fever was the most common clinical symptom history, followed by joint pain in this study. From the clinical symptoms obtained, the clinical symptoms of chikungunya and dengue fever are somewhat difficult to distinguish, so misdiagnosis often occurs; where chikungunya is diagnosed as dengue fever, it's just that complaints of joint pain are more dominant in chikungunya and adults are more predominant in chikungunya, while in dengue fever joint pain is less and mainly affects children. The dominant pain symptom in the joints makes it possible to distinguish chikungunya from dengue hemorrhagic fever by the clinician in helping the diagnosis.

Chikungunya infection generally causes a sudden attack and is characterized by sudden fever >38.5 ºC, joint pain, rash, and other flu-like symptoms such as headache and fatigue (16). Carson et al., in their research on the chikungunya outbreak in Western Jamaica, stated that fever and joint pain are the most common symptoms in chikungunya sufferers (17). Joint pain experienced by chikungunya sufferers generally occurs in the joints in the elbows, wrists, fingers, knees, and ankles (5). Joint pain is due to fibroblasts and connective tissue, commonly found in the joint area and susceptible to chikungunya virus infection. In some cases, symptoms of joint pain can last for months (5,18).

IgM is detected in the blood a few days after the onset of symptoms and persists for up to 3 months, while IgG is detected around day four after the onset of symptoms and may persist for years. As with most arboviruses, many infected people experience only mild or no symptoms (8). Viral exposure and immunological factors such as cytokines, chemokines, or molecular factors elicited by post-CHIKV infection modulate the IgG response qualitatively and quantitatively.

The importance of antibodies that serve as a critical barrier against viral infection was also demonstrated for CHIKV (19).

Although several mosquito-borne viral infections have been reported in Indonesia, these diseases have received little attention as a public health threat for decades. The co-circulation of mosquito-borne viral infections is a public health concern, as they often cause fevers not recognized by clinicians, especially in areas with inadequate laboratory capacity (20). Accurate and up-to-date epidemiological data on clinical cases of these mosquito-borne viral infections are limited in many parts of Indonesia. This is most likely because the disease is asymptomatic, and symptoms are generally mild and non-specific if it does occur. They thus may not be detected or reported at healthcare facilities. This study's findings provide important information regarding chikungunya epidemiology and intervention in Makassar.

This study has several limitations. Firstly, the sample size was small, which may have impacted the accuracy of the results. Second, interviewing participants with less knowledge of chikungunya may introduce recall bias and may also affect the level of symptomatic disease, especially if a person was infected and became symptomatic in another epidemic area long before this study was conducted.

CONCLUSIONS

The findings of this study provide evidence of CHIKV exposure in Makassar city. The presence of IgG antibodies to microbes cannot be used as an indicator of infection, but it is important to know the level of population exposure.

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Conflict of Interest

The authors declare no conflicts of interest. The funder had no role in the study's design, the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to publish the results.

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