TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2017) 47: 1161-1164

doi:10.3906/sag-1604-36

A study of the chikungunya virus in humans in Turkey

Tuğba ATALAY[1], Sedat KAYGUSUZ[2,*], Ahmet Kürşat AZKUR[3]

[1]Department of Medical Microbiology, Faculty of Medicine, Kırıkkale University, Kırıkkale, Turkey
[2]Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Kırıkkale University, Kırıkkale, Turkey
[3]Department of Virology, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale, Turkey

Received: 09.04.2016 • **Accepted/Published Online:** 31.03.2017 • **Final Version:** 23.08.2017

Background/aim: The chikungunya virus (CHIKV) is a mosquito-borne disease and has recently been causing explosive outbreaks. The CHIKV has spread throughout all continents. Although the first chikungunya case imported from India to Turkey was reported in 2012, there is no detailed epidemiologic study in Turkey yet. The aim of this study was to investigate the seroprevalence of the CHIKV in Turkey.

Materials and methods: ELISA was used to screen 500 random serum samples of healthy people collected from Kırıkkale, which is located in central Anatolia in Turkey. The results were verified by indirect immunofluorescence test (IIFT).

Results: The results showed that 0.4% samples were positive for CHIKV. In the verification study with IIFT, CHIKV IgG type antibodies were defined as negative. To the best of our knowledge, this is the first serological study on the CHIKV in Turkey.

Conclusion: Further studies are needed to elucidate the epidemiological situation in patients that have fever and arthritis.

Key words: Chikungunya virus, ELISA, serology, Turkey

1. Introduction

The chikungunya virus (CHIKV) is a single-stranded, positive sense RNA virus that is a member of the genus *Alphavirus* of the family *Togaviridae*. The CHIKV is transmitted primarily by *Aedes aegypti* and *Aedes albopictus* mosquitoes (1). CHIKV infection led to more than 6 million confirmed cases worldwide (2). CHIKV has a wide geographic distribution including North and South America, Europe, Asia, the Pacific Islands, and Africa since the virus was first described in Tanzania in 1952 (3,4). A female patient who came from India to Turkey with symptoms of fever, arthralgia, and rashes was the first case of laboratory-confirmed CHIKV infection in Turkey (5). Clinical symptoms of CHIKV infection include high fever, fatigue, backache, headache, and polyarthralgia. Polyarthralgia is the most characteristic and common symptom of infection and also the origin of the name of the disease "chikungunya," a local term in the Makonde language that means "disease that bends up the joints". In addition to these clinical manifestations, tenosynovitis, swollen joints, macular or maculopapular skin rashes, myalgia, nausea, vomiting, and diarrhea can be observed in CHIKV-infected patients (3,6). Differential diagnosis of CHIKV infection from dengue virus infection could be based on clinical manifestations and laboratory features. Fever (over 39 °C), arthritis, arthralgia, rash, and lymphopenia are more significant in a CHIKV infection. While hemoconcentration does not occur in CHIKV infection, it is present in 70%–100% of dengue virus-infected patients (2). Diagnosis of CHIKV infection is based on molecular detection of a viral genome and/or serological detection of virus-specific antibodies. RT-PCR and real-time RT-PCR (7–9) can be used for molecular detection while serological diagnostic tests include ELISA, immunofluorescence assay, and rapid immunochromatographic test (10–13). The aim of this study was to screen for possible exposure to CHIKV infections in humans using ELISA and IIFT in the city of Kırıkkale, which is located in the central Anatolia region of Turkey.

2. Materials and methods

2.1. Samples

Blood samples were taken from 500 healthy, randomly selected volunteer blood donors who live in Kırıkkale (39°50′N; 33°31′E; altitude 700 m) through July–November

* Correspondence: sedatkaygusuz@msn.com

2015 (Table). Samples from volunteers, who were accepted as healthy blood donors after the assessment of a donor questionnaire, were collected with their informed consent. The study was approved by the Ethic Review Committees of the Faculty of Medicine, Kırıkkale University (Date 11.05.2015/Decision number: 12-02). Blood samples were collected into tubes without an anticoagulant for the separation of serum, allowing for clotting at room temperature, and centrifuged at 2000 rpm for 10 min. Sera were collected and kept at −80 °C until use.

2.2. Detection of anti-CHIKV IgG

A semiquantitative anti-chikungunya IgG ELISA test (Catalog number EI 293a-9601G Euroimmun) was performed according to the manufacturer's instructions. Reference controls included positive, negative, and blank samples. The Ab% values were calculated using the following formula: Ratio = Extinction of the control samples or patient samples / Extinction calibrator. A serum sample was considered positive if Ab% was ≥1.1, while a sample was considered negative if Ab% was ≤0.8. An Ab% value of 0.8–1.1 indicated a borderline sample. Blood samples were investigated in terms of IgG type antibodies by IIFT with a commercial kit (Catalog number FI293a-1010G, Euroimmun). The manufacturer confirms the CHIKV IgG IIFT test's sensitivity as 96.7% and specificity as 100%.

3. Results

A total of 500 serum samples from healthy people were tested by ELISA. Two out of the 500 (0.4%) sera were positive for the CHIKV. The positive results were obtained from a woman aged 79 who is a housewife from Keskin District (OD value 1.385) and a man aged 55 who is a farmer from Balışeyh District (OD value 1.242). Four out of the 500 (0.8%) sera were borderline. In the validation study with IIFT, CHIKV IgG type antibodies were defined as negative. To the best of our knowledge, this is the first serological study on the CHIKV in Turkey.

4. Discussion

The CHIKV causes mosquito-borne disease of key public health importance in tropical and subtropical countries and the health and economic burden due to this virus is enormous. The La Reunion outbreak cost the French authorities millions in lost productivity, and over €43 million in direct medical costs (14).

CHIKV probably first emerged as a human pathogen in the 18th century and certainly reemerged periodically with relatively low prevalence in Africa but also in Asia, with the earliest outbreak in the Philippines in 1954. CHIKV has been reported in many countries in Asia, America, and Africa after an epidemiologic silent period as a neglected tropical disease (2). The reemergence of CHIKV as an urban epidemic was described in Kinshasa, Democratic Republic of the Congo, in 2000 (15). A chikungunya epidemic occurred in several states of India in 2005–2006, affecting about 1.3 million people. The Pan American Health Organization recorded 776,000 cases and 152 deaths attributed to CHIKV infection in 33 countries in 2014 (http://www.paho.org/chikungunya). CHIKV infection has been identified in nearly 80 countries across 5 continents and caused more than 6 million confirmed cases (2). While CHIKV imported cases into Northern Italy were very low between 2011 and 2013, the number of imported cases has increased significantly since 2014 (16). Besides Italy, Turkey also has a coast on the Mediterranean Sea. Chikungunya infection is transmitted between humans by Aedes mosquitoes. Presence of Aedes mosquitoes was reported in Turkey as well (17–19). The first chikungunya case imported from New Delhi, India,

Table. Kırıkkale and its population and sampling data for Kırıkkale.

Province	Number of samples	Male	Female	≤18 year olds
City center	364	134	230	37
Bahşılı	13	7	6	5
Balışeyh	11	6	5	2
Celebi	4	1	3	0
Delice	16	7	9	0
Karakeçili	7	4	3	0
Keskin	33	6	27	9
Sulakyurt	13	4	9	7
Yahşihan	39	18	21	2
Total	500	187	313	62

to Ankara, Turkey, was reported in 2012. However, this is the first seroepidemiological study in Turkey and CHIKV specific antibodies were reported for the first time in Kırıkkale in Turkey.

A concern was that the commercial kit employed may detect the antibodies fighting against other Semliki Forest virus antigenic complex viruses. The CHIKV is part of the Semliki Forest virus antigenic complex that also includes O'Nyong Nyong, Mayaro, and Ross River viruses. It was evaluated, including four CHIKV serologic diagnostic tests. The Euroimmun ELISA, which was used this study, had a specificity of 95% (IgG) and a sensitivity of 88% (IgG). The Euroimmun ELISA test was evaluated as to whether it might detect Mayaro and O'Nyong-Nyong virus and positivity was found (20). Until day 7 after disease onset genome detection by RTPCR is recommended for diagnosis. IgM and IgG antibodies can be detected as early as 3–6 days after the onset of clinical symptoms. It was reported that IIFT is more sensitive than ELISA. For the commercial IgG assay the specificity was 100% and the sensitivity 95.4% (10)

In conclusion, this research is the first CHIKV seroprevalence study in Turkey. Later studies should focus on viruses in mosquitoes. In addition, the proximity of mosquito vector breeding areas in the Kızılırmak is an important risk factor for other diseases transmitted by the same vector as well as CHIKV. For the prevention and control of vector transmissions, municipalities need to be mobilized. CHIKV fever should be kept in mind when clinicians encounter patients who have fever and arthralgia and a history of visits to endemic places.

Acknowledgment
This study was supported by the Scientific Research Project Coordination Unit of Kırıkkale University (KUBAP) with project number 2015/12.

References

1. Vega-Rúa A, Lourenço-de-Oliveira R, Mousson L, Vazeille M, Fuchs S, Yébakima A, Gustave J, Girod R, Dusfour I, Leparc-Goffart I et al. Chikungunya virus transmission potential by local Aedes mosquitoes in the Americas and Europe. PLoS Negl Trop Dis 2015; 9: e0003780. doi: 10.1371/journal.pntd.0003780.
2. Petitdemange C, Wauquier N, Vieillard V. Control of immunopathology during chikungunya virus infection. J Allergy Clin Immunol 2015; 135: 846-855.
3. Ross RW. The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. J Hyg (Lond) 1956; 54: 177-191.
4. CDC; http://www.cdc.gov/chikungunya/geo/index.html.
5. Yağcı Çağlayık D, Uyar Y, Korukluoğlu G, Ertek M, Unal S. An imported Chikungunya fever case from New Delhi, India to Ankara, Turkey: the first imported case of Turkey and review of the literature. Mikrobiyol Bul 2012; 46: 122-128.
6. Thiberville SD, Boisson V, Gaudart J, Simon F, Flahault A, de Lamballerie X. Chikungunya fever: a clinical and virological investigation of outpatients on Reunion Island, South-West Indian Ocean. PLoS Negl Trop Dis 2013; 7: e2004. doi: 10.1371/journal.pntd.0002004.
7. Hasebe F, Parquet MC, Pandey BD, Mathenge EG, Morita K, Balasubramaniam V, Saat Z, Yusop A, Sinniah M, Natkunam S et al. Combined detection and genotyping of Chikungunya virus by a specific reverse transcription-polymerase chain reaction. J Med Virol 2002; 67: 370-374.
8. Santhosh SR, Parida MM, Dash PK, Pateriya A, Pattnaik B, Pradhan HK, Tripathi NK, Ambuj S, Gupta N, Saxena P et al. Development and evaluation of SYBR Green I-based one-step real-time RT-PCR assay for detection and quantification of Chikungunya virus. J Clin Virol 2007; 39: 188-193.
9. Ho PS, Ng MM, Chu JJ. Establishment of one-step SYBR green-based real time-PCR assay for rapid detection and quantification of chikungunya virus infection. Virol J 2010; 7: 13.
10. Litzba N, Schuffenecker I, Zeller H, Drosten C, Emmerich P, Charrel R, Kreher P, Niedrig M. Evaluation of the first commercial chikungunya virus indirect immunofluorescence test. J Virol Methods 2008; 149: 175-179.
11. Yap G, Pok KY, Lai YL, Hapuarachchi HC, Chow A, Leo YS, Tan LK, Ng LC. Evaluation of Chikungunya diagnostic assays: differences in sensitivity of serology assays in two independent outbreaks. PLoS Negl Trop Dis 2010; 4: e753. doi: 10.1371/journal.pntd.0000753.
12. Rianthavorn P, Wuttirattanakowit N, Prianantathavorn K, Limpaphayom N, Theamboonlers A, Poovorawan Y. Evaluation of a rapid assay for detection of IgM antibodies to chikungunya. Southeast Asian J Trop Med Public Health 2010; 41: 92-96.
13. Okabayashi T, Sasaki T, Masrinoul P, Chantawat N, Yoksan S, Nitatpattana N, Chusri S, Morales Vargas RE, Grandadam M, Brey PT et al. Detection of chikungunya virus antigen by a novel rapid immunochromatographic test. J Clin Microbiol 2015; 53: 382-388.
14. Rolph MS, Zaid A, Mahalingam S. Salivary transmission of the chikungunya arbovirus. Trends Microbiol 2016; 24: 86-87.
15. Burt FJ, Rolph MS, Rulli NE, Mahalingam S, Heise MT. Chikungunya: a re-emerging virus. Lancet 2012; 379: 662-671.
16. Rossini G, Gaibani P, Vocale C, Finarelli AC, Landini MP. Increased number of cases of Chikungunya virus (CHIKV) infection imported from the Caribbean and Central America to northern Italy, 2014. Epidemiol Infect 2016; 1-5.

17. Alten B, Bellini R, Caglar SS, Simsek FM, Kaynas S. Species composition and seasonal dynamics of mosquitoes in the Belek region of Turkey. J Vector Ecol 2000; 25: 146-154.

18. Akiner MM, Demirci B, Babuadze G, Robert V, Schaffner F. Spread of the invasive mosquitoes *Aedes aegypti* and *Aedes albopictus* in the Black Sea Region increases risk of chikungunya, dengue, and zika outbreaks in Europe. PLoS Negl Trop Dis 2016; 10: doi: 10.3201/eid2012.141269.

19. European Centre for Disease Prevention and Control (ECDC). Mosquito maps. http://ecdc.europa.eu/en/healthtopics/vectors/vector-maps/Pages/VBORNET_maps.aspx (accession date: 20.02.2017).

20. Prat CM, Flusin O, Panella A, Tenebray B, Lanciotti R, Leparc-Goffart I. Evaluation of commercially available serologic diagnostic tests for chikungunya virus. Emerg Infect Dis 2014; 20: 2129-2132.