Seroepidemiology of Chikungunya Fever in Dhaka, Bangladesh: A Cross-sectional Study

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Chikungunya fever (CHIKF) is a mosquito-borne febrile illness caused by the Chikungunya virus (CHIKV). Bangladesh has documented several outbreaks of CHIKF since it was first reported in 2008. The latest CHIKF outbreak occurred in the Dhaka in 2017. In this study, a serosurvey of the 2017 outbreak was conducted during its peak. The study involved the assessment of CHIKV immunoreactions among participants suffering from CHIKF-related symptoms. One hundred blood samples were collected from patients suffering from CHIKF-associated symptoms and were subsequently tested for the presence of anti-CHIKV IgM and/or IgG antibodies. Data based on clinical symptoms and the demographics of the participants were recorded and analyzed. Seventy-four percent of the studied patients (n = 100) possessed anti-CHIKV antibodies. Among this seropositive group (n = 74), almost 62% contained anti-CHIKV IgM and IgG antibodies, whereas 10% contained only anti-CHIKV IgM antibodies indicating recent infection. Anti-CHIKV IgG antibodies were found in 28% of patients. Among the symptoms examined, polyarthralgia showed a highly significant relationship (P < 0.001), whereas high fever and the presence of rash demonstrated a significant association with CHIKV seropositivity (P < 0.05). Discoveries made on this research can better help public health officials to gain a comprehensive insight into the seroepidemiology of the condition based in the city and maintain constant vigilance against any future outbreak.

Keywords: Chikungunya Virus, Seroepidemiology, Dhaka, Bangladesh.

Introduction

The Chikungunya virus (CHIKV) is an alphavirus of the Togaviridae family first discovered in 1952-1953 in Tanzania¹. The virus is transmitted by the female Aedes mosquitoes, mainly the A. aegypti and A. albopictus¹,². The most prominent symptoms of the disease are high fever, polyarthralgia, myalgia, headache and maculopapular rash. Other notable symptoms may include nausea and vomiting. Fever and rash last for a very short time, but the pain in the joint is incapacitating and persistent. CHIKV infection of a population lacking herd immunity usually produces a massive epidemic affecting an enormous number of people. The interactions of some factors bring about this occurrence: vulnerable human populations, availability of the vectors A. aegypti and A. albopictus and viral mutations that augment its infectivity².

CHIKV infection can be diagnosed based on clinical, epidemiological and laboratory findings. The sudden appearance of high-grade fever and debilitating pain in the joint are the major criteria for clinical diagnosis. However, in parts of the world where Chikungunya, Dengue and Zika viruses co-exist, clinical diagnosis of CHIKF can be difficult⁴. Diagnosis using epidemiological data can be done by the use of the suspected patient’s travel history to areas that going through a CHIKF outbreak. The laboratory diagnosis of CHIKF involves isolation and characterization of the virus, detection of viral nucleic acid and antibody detection. Diagnosis of CHIKF using molecular techniques such as RT-PCR, RT-LAMP, qRT-PCR are the most widely acceptable due to their high specificity and sensitivity. However, serological tests aimed at detecting serum IgM and/or IgG are the most popular methods of CHIKF diagnosis as they are the cheapest of all the diagnostic techniques and most convenient to conduct on patients.⁷

From the standpoint of epidemiology, Chikungunya fever (CHIKF) outbreaks tend to disappear for a sizable period of time from a particular part of the world before reemerging again. For instance, the CHIKF outbreak around the Indian Ocean in 2005 have occurred nearly after three decades. In Asia, the first outbreak was reported in Thailand in 1958. Since then, the disease has been reported in many countries of Southeast Asia. India first reported the CHIKF outbreak in 1964 in its southern regions. Being the country adjacent to India, Bangladesh documented the first outbreak of the disease in the year 2008 at Rajshahi and Chapainawabganj, the border-districts with India. Since then, the disease has behaved as a re-emergent disease in the country with outbreaks occurring in 2011 at the sub-district of Dohar, Dhaka and again, in 2012 in the village of Palpapara of Tangail district.¹³

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Dhaka, the capital of Bangladesh, experienced the latest outbreak of CHIKF in the monsoon season of 2017. This epidemic was widely covered by the country’s mainstream media\textsuperscript{14}. A government organization, Institute of Epidemiology, Disease Control and Research (IEDCR), actively monitored the epidemic. Based on their newsletter on CHIKV, the epidemic peaked between early May and late July of 2017. Their newsletter also reported that 12060 out of 13814 people with CHIKV-related symptoms had visited three of the most prominent public hospitals along with IEDCR itself for clinical aid. Moreover, there was no alert of any other arbovirus-related diseases in the country at that time\textsuperscript{15}. A few scientific reports studying this Dhaka epidemic of 2017 have already been published \textsuperscript{7,16,17}. We believe findings of the current research will complement these previously published documents and add to the knowledge that already prevails on CHIKF seroepidemiology in Dhaka. Subsequently, identifying and controlling the risk factors of the infections might be much easier for health workers and policymakers to maintain a surveillance system against any CHIKF epidemics in the future.

Materials and Methods

Study framework
This cross-sectional epidemiological study was carried out in Dhaka, Bangladesh from July 17, 2017 to August 5, 2017. The study comprised individuals who were suffering from CHIKF-related symptoms and came to the Ad-Din Hospital for a Chikungunya infection test. A total of 100 participants were selected based on a combination of inclusion and exclusion criteria. The inclusion criteria included those who were suffering from CHIKF-related symptoms such as high fever and joint pain accompanied by rash, severe headache. The exclusion criteria involved screening out individuals who had a history of suffering from dengue fever, arthralgia and arthritis. Each eligible respondent was interviewed personally after they were detailed on the process and purpose of the study and they provided their verbal consent. Since the patient came to the hospital for Chikungunya diagnosis, they already provided their blood to the hospital authority for the test. We did not have to physically puncture them for blood collection solely for our research, rather the blood specimen in our research was from the blood that was collected for the diagnosis purpose. A structured interview schedule was filled out based on the interview. This interview schedule consisted of two sections: the respondent’s demographic data and data on the CHIKF-related signs and symptoms experienced. Afterwards, every respondent was assigned a unique identification number (ID), which was later used for serodiagnosis and data analysis.

Sample collection and processing
All 100 patients were detailed about the purpose and the process of the study. Blood specimens were collected, serum was separated and extracted as described by Mahbub et al.\textsuperscript{18}. Three milliliters of blood were taken from each participant in a sterile glass test tube. The test tubes were allowed to rest at a slanted position for 1 hour for the blood to clot. Serum, collected at the upper level of the test tube, was transferred to a sterile Eppendorf tube with a Pasteur pipette. These tubes were centrifuged (Eppendorf, Germany; Model: 22331) at 3000 rpm for 10 minutes so that the remaining blood cells settled down. Clear serum was then transferred to another sterile Eppendorf tube and stored at -20°C until serodiagnosis.

Serodiagnosis
Every serum sample was tested qualitatively for the presence of anti-CHIKV IgG and/or IgM antibodies using a commercially available immunochromatographic assay kit called Standard Q Chikungunya IgM/IgG Test (Product Cat. No. 09CHI20D, Manufacturer: SD BIOSENSOR, Republic of Korea)\textsuperscript{19}. The conduction of the tests and the interpretation of the results were carried out strictly following the instruction manual provided with the kit.

Processing and analysis of data
Frequency distributions of the respondents based on their serostatus of anti-CHIKV antibodies, their clinical profiles and socio-demographic factors were made. Chi-square tests were employed as a measure of association. Subsequently, a multiple logistic regression model was set up choosing variables (P < 0.25) from the Chi-square tests to avoid missing essential covariates in the model\textsuperscript{20}. The discriminatory performance of the model was tested by using the “Receiver Operating Characteristic (ROC) Curve” and the area under the ROC curve was calculated\textsuperscript{21}. A variable that yielded a P < 0.05 was considered statistically significant while one that emerged with a P < 0.001 was considered statistically highly significant. Data of the participants were tabulated and analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). For the evaluation of the severity of pain, a 10-point numerical rating scale (NRS) (Table 4) was used\textsuperscript{22}. Ratings were categorized into mild (scores between 1 and 3), moderate (scores between 4 and 6), and severe (scores between 7 and 10). Follow-ups were made with the participants in intervals of 1 month for 3 months to check if they still experienced the pain in their joints.

Results

Demographic characteristics of participants
Of the 100 individuals who were randomly chosen and tested serologically for the presence of anti-CHIKV IgM and/or IgG, 74 (74%, n = 100) individuals exhibited presence of IgM and/or IgG in their blood. This group was categorized as “seropositive,” while the remaining 26 respondents were classified as seronegative. The 74% of the seropositive group was further broken down into three different classes: people having IgM only (10%, n = 74), people having IgG only (28%, n = 74), and people having both IgG and IgM (62%, n = 74) (Figure 1).
A look into the socio-demographic status of the studied patients (Table 1) depicted that 71% (n = 100) participants were male while the remaining 29% (n = 100) were female. The male to female ratio was calculated at 2.45:1. The mean age of the studied patients was 30.64 (SD = 14.90). Children constituted 26% of the surveyed patients, while 69% were adults and 5% were elderly. Incidence rate was highest among adults aged between 18 and 59 years (73%), followed by children (63%) and elderly (4%).

**Table 1. Socio-demographic profile of the patients**

| Socio-demographic characteristics | Serostatus of anti-CHIKV antibodies | Total |
|-----------------------------------|-------------------------------------|-------|
|                                   | Seropositive (%) | Seronegative (%) |     |
| All                               | 74 (100) | 26 (100) | 100 |
| Age group (in years)              |           |           |     |
| Children (<18)                    | 17 (23.0) | 9 (34.6) | 26  |
| Adults (18-59)                    | 54 (73.0) | 15 (57.7)| 69  |
| Elderly (>59)                     | 3 (4.0)   | 2 (7.7)  | 5   |
| Gender                            |           |           |     |
| Male                              | 50 (67.6) | 21 (80.8)| 71  |
| Female                            | 24 (32.4) | 5 (19.2) | 29  |
| Marital Status                    |           |           |     |
| Married                           | 36 (48.6) | 5 (19.2) | 41  |
| Unmarried                         | 38 (51.4) | 21 (80.8)| 59  |
| Education                         |           |           |     |
| Uneducated                        | 5 (6.8)   | 0 (0.0)  | 5   |
| Primary                           | 8 (10.8)  | 2 (7.7)  | 10  |
| Secondary                         | 43 (58.1) | 17 (65.4)| 60  |
| Graduated                         | 18 (24.3) | 7 (26.9)| 25  |
| Occupation                        |           |           |     |
| Service holder                    | 33 (44.6) | 3 (11.5) | 36  |
| Student                           | 35 (47.3) | 20 (76.9)| 55  |
| Housewife                         | 4 (5.4)   | 2 (7.7)  | 6   |
| Retired                           | 2 (2.7)   | 1 (3.8)  | 3   |

Analysis of clinical and behavioral traits of the participants

The most prominent symptoms observed in the current study were high fever, polyarthralgia, rash, headache, myalgia, and vomiting (Table 2). High fever (P = 0.002) and rash (P = 0.008) demonstrated significant relationship with the presence of anti-CHIKV IgM and/or IgG. In the multiple logistic regression analysis (Table 3), people with high fever (≥ 103°F) were about 1.9 times (CI: 0.393 – 9.249) more likely to be seropositive against CHIKV than that of people with fever below 103°F. Likewise, someone with rash was about 1.8 times (CI: 0.525 – 6.165) more likely to possess anti-CHIKV antibodies than that of the people having no rash. Meanwhile, the relationship between respondents having polyarthralgia and being immunopositive for anti-CHIKV IgM and/or IgG was highly significant at P < 0.001. In this case, the likelihood for someone with joint pain to be immunopositive for anti-CHIKV antibodies was about 11 times (CI: 1.792 – 67.042) higher than that of someone with no pain. Other clinical symptoms such as headache, vomiting, and myalgia did not show any significant relationship. In addition to the clinical manifestations, some behavioral attributes that could have contributed to the infection in the immunopositive group were

**Fig.1. Classification of the seropositive group based on the presence of anti-CHIKV Immunoglobulins.**

**Table 2. Clinical profile and behavioral variables of the respondents in Dhaka CHIKF outbreak of 2017**

| Variables                              | Serostatus of anti-CHIKV antibodies | P value |
|----------------------------------------|-------------------------------------|---------|
| All                                    | Seropositive (%) | Seronegative (%) |     |
| Signs and Symptoms exhibited           | 74 (100) | 26 (100) |     |
| High fever                             |           |           |     |
| <103 °F                                | 9 (12.2) | 11 (42.3) | 0.001* |
| ≥103 °F                                | 65 (87.8) | 15 (57.7) |     |
| Polyarthralgia                         |           |           |     |
| No                                     | 5 (6.8) | 13 (50.0) | 0.000** |
| Yes                                    | 69 (93.2) | 13 (50.0) |     |
| Rash                                   |           |           |     |
| No                                     | 29 (39.2) | 18 (69.2) | 0.008* |
| Yes                                    | 45 (60.8) | 8 (30.8)  |     |
| Headache                               |           |           |     |
| No                                     | 30 (40.5) | 11 (42.3) | 0.875 |
| Yes                                    | 44 (59.5) | 15 (57.7) |     |
| Vomiting                               |           |           |     |
| No                                     | 51 (68.9) | 21 (80.8) | 0.247 |
| Yes                                    | 23 (31.1) | 5 (19.2)  |     |
| Myalgia                                |           |           |     |
| No                                     | 27 (36.5) | 11 (42.3) | 0.599 |
| Yes                                    | 47 (63.5) | 15 (57.7) |     |
| Selected behavioural variables:        |           |           |     |
| Travelled outside Dhaka before illness | 64 (86.5) | 25 (96.2) | 0.175 |
| Yes                                    | 10 (13.5) | 1 (3.8)   |     |
| Use of mosquito control measures       |           |           |     |
| No                                     | 41 (55.4) | 17 (65.4) | 0.375 |
| Yes                                    | 33 (44.6) | 9 (34.6)  |     |
| Household members with similar signs and symptoms | 34 (45.9) | 20 (76.9) | 0.006* |
| Yes                                    | 40 (54.1) | 6 (23.1)  |     |
| Availability of stagnant water near the residence | 0 (0.0) | 0 (0.0) | - |
| Yes                                    | 74 (100.0) | 26 (100.0) |     |
also investigated. About 46% (n = 74) of the seropositive patients informed that they had at least one household member who exhibited similar CHIKF-related symptoms. From a statistical point of view, the relationship between a person immunopositive for anti-CHIKV antibodies and having at least one household member with similar ailments was significant at a P-value of 0.006.

Table 4 depicts an in-depth look at the polyarthralgia profile of the seropositive group of the studied patients. Sixty-nine seropositive participants (93.3%, n = 74) complained of pain in various joints of their bodies during the duration that they were ill. To understand the intensity of pain they experienced, the study group was asked to rate the severity of their pain. Forty-two seropositive individuals (56.8%, n = 74) rated their pain in the severe class. This was highest, followed by 28 individuals (37.8%, n = 74) whose pain was moderately severe. Seventy-two respondents (97.3%, n = 74) informed that they had taken some form of medications to reduce the pain but to no avail.

The multiple logistic regression model was evaluated using the ROC curve. The area under the ROC curve (Figure 2) was 0.881

| Variables                                      | Serostatus of anti-CHIKV antibodies |
|------------------------------------------------|-------------------------------------|
|                                                | Seropositive (%) | Seronegative (%) |
| All 74 (100)                                   | 26 (100)          |                  |
| Duration of pain                               |                     |                  |
| No arthralgia                                  | 5 (6.8)            | 13 (50.0)        |
| Less than 7 days                               | 13 (17.6)          | 5 (19.2)         |
| Acute (7-14 days)                              | 10 (13.5)          | 4 (15.4)         |
| Subacute (up to 90 days)                       | 41 (55.4)          | 4 (15.4)         |
| Chronic (more than 90 days)                    | 5 (6.8)            | 0 (0.0)          |
| Pain intensity                                 |                     |                  |
| No pain                                        | 2 (2.7)            | 5 (19.2)         |
| Mild (NRS scores of 1 – 3)                     | 2 (2.7)            | 3 (11.5)         |
| Moderate (NRS scores of 4 – 6)                 | 28 (37.8)          | 14 (53.8)        |
| Severe (NRS scores of 7 – 10)                  | 42 (56.8)          | 4 (15.4)         |
| Analgesics used                                |                     |                  |
| No 2 (2.70)                                    | 7 (26.92)          |                  |
| Yes 72 (97.30)                                 | 19 (73.08)         |                  |
Fig. 2. ROC curve for the Logistic regression model showing an area of 0.881 (CI: 0.807 - 0.956).

Discussion

Dhaka is a city with a population density of 23,234 people per square kilometer. The climate of the city is generally hot, wet, and humid, with distinct monsoon periods. About 87% of the annual average rainfall of 2,123 mm occurs between May and October. Over one-third of the population is poor and lives in densely populated slums. During the monsoon, water bodies scattered throughout the city rise in levels. Garbage, such as plastic containers and rubber tires, holds clear water, providing suitable growth sites for mosquito larvae. On top of that, Dhaka witnessed an alarming level of rainfall in the year 2017 during the CHIKF epidemic. Both the CHIKF epidemic and the high rainfall were covered by the country’s news media. Also, as calculated by Dhar-Chowdhury et al., the Breteau Index (BI or number of A. aegypti infested containers per 100 houses inspected) in Dhaka was as high as in between the range of 52.0 and 63.4. Therefore, it is not surprising that the CHIKV vector is so widespread in most of the city’s administrative units.

The overall seroprevalence of anti-CHIKV IgM and/or IgG in this study was 74% (n = 100). Out of these, 10% (n = 74) had IgM only while 62% had both IgM and IgG, indicating recent infections in the individuals. On the other hand, 28% of the immunonegative group (n = 74) had only IgG in their serum indicating past infections. The overall seroprevalence of 74% was in close agreement to that reported by Rashid et al. who investigated the same outbreak.

The investigated patients of the present study exhibited the typical symptoms of CHIKV infection, including fever, polyarthralgia, rash, headache, vomiting, myalgia. Like most reports, 59.5% (n = 74) of the seropositive patients reported that the illness began with sudden onset of high fever followed by debilitating joint pain. In contrast, Hossain et al. and Anwar et al. who conducted their own separate investigations of the same outbreak, reported that the major proportion of the patients in their study experienced pain in joints as the initial symptom. High fever and arthralgia together were documented in 93.2% (n = 74) of the seropositive respondents confirmed with anti-CHIKV antibody. Fever and arthralgia can therefore be considered the hallmark of this study. This finding also conforms with previous epidemics in the Réunion Island, Panama, Suriname, Singapore and Dhaka. Four participants (5.41%, n = 74) with anti-CHIKV antibodies reported to have experienced no arthralgia but having the other typical symptoms of CHIKF. A similar finding has also been reported by Osterrieth et al.

About 97.3% of the studied individuals suffered from high fever (P = 0.001; Table 2) at an average highest body temperature of 103.6°F (SD = 1.30) that lasted for 4.86 days (SD = 2.95). Hossain et al. reported this very same finding in their study. Remarkably, 5.4% (n = 74) of the seropositive group informed that their body temperature spiked as high as 106°F, whereas another 10.8% said theirs reached 105°F. Furthermore, the multiple logistic regression model (Table 3) demonstrated that participants recording the highest body temperature greater than or equal to 103°F were at 1.9 times higher odds of being seropositive for anti-CHIKV antibody.

Polyarthralgia was most deeply investigated because of the adverse impact it had on the quality of daily life of the patients. From a statistical standpoint, the relationship between seropositivity for anti-CHIKV antibody and suffering from arthralgia was highly significant at P < 0.001 (Table 2). In the multiple logistic regression analysis (Table 3), a person with joint pain was about 11 times more likely to be positive for anti-CHIKV IgM and/or IgG than a person who did not have pain in the joints. The patients were further classified (Table 4) based on the duration for which they suffered. Those who were immunonegative for anti-CHIKV antibodies, 41 (55.4%, n = 74) suffered for durations ranging from 15 to 89 days, while 5 participants (6.8%, n = 74) had the pain in the chronic stage with the duration of pain exceeding 90 days. As it is common that arthralgia associated with CHIKV infection has little or no response to analgesics, we inquired every participant if they took any medication for the pain. Locally available pain medicines were used, but not one of the respondents had any relief from the pain. Contrarily, they complained about hindered daily life; some patients even informed this pain was the worst they had ever suffered from. This same complaint was registered by subjects of the epidemics in Madras City and Réunion Island. As a result, we tried to evaluate the severity of pain experienced by our respondents based on a 10-point numerical rating scale (NRS) (Table 4). Forty-two (56.8%, n = 74) of the seropositive respondents fell in the category of severe joint pain. For example, 10 patients (13.5%, n = 74) had the pain in the acute stage, while 5 (6.8%, n = 74) had the pain in the subacute stage. The mean pain score (NRS) (Table 4).
7 – 10), and 28 (37.8%) recorded moderate pain intensity (NRS score 4 – 6). As corroborated by our study, the overall measurement of pain intensity was also generally high in other similar seroepidemiology of CHIKV, especially those conducted in Dhaka in 2017. The reason behind prolonged suffering from pain in the joints is still poorly understood. Studies have speculated that the persistence of this particular symptom of CHIKF could be due to the inflammatory response of the host, the presence of viral products in joint tissues and macrophages for long periods of time and the involvement of some auto-immune responses in pathogenesis.

Apart from fever and arthralgia, severe headaches, skin rash, and vomiting were other notable symptoms observed. Among these, rash development had statistical significance with a P-value of 0.008 (Table 2). The logistic regression model gave a person with rash on their skin 1.8 times (Table 3) more chance of being seropositive for anti-CHIKV antibodies.

As mentioned earlier, Dhaka provides a favorable breeding ground for CHIKV vectors. In addition, the year 2017 produced atypically high rainfall. We tried to see if this aspect had any effect on the outbreak of CHIKF. Respondents were asked if they had any source of stagnant water surfaces around their area of residence. This could have been anything from a puddle of clean water or a potted plant at home. As Dhaka is littered with waterlogged streets during seasons of high rainfall, all 100 of the participants in our research informed that they had some form of stagnant waterbodies near them. Although this was expected, it could not be analyzed with a statistical tool (Table 2) due to the lack of variability in data.

Another interesting aspect that caught our attention was that 54.1% of the seropositive individuals in our target group noticed similar CHIKF symptoms in other members of their household. People who claimed to have witnessed other members of their household exhibiting CHIKF-like symptoms were at greater odds to be seropositive for anti-CHIKV antibodies (P = 0.006; O.R: 5.905, CI: 1.592-21.906). Although the other members of the households could not be included in the study, this finding, however appeared to be coherent with the behavioral pattern of Aedes vectors. The vector is known to live in close proximity to people and bite several people at a time for the same blood meal. They also have a flight range of merely a hundred meters. These characteristics of A. aegypti could point to the fact that people living in the same household with symptoms of CHIKF are prone to be bitten by the same infected mosquito so that they succumb to the disease at the same time.

The clinical profile demonstrated by the participants of this study conformed with several previously documented CHIKF outbreaks worldwide. However, this study is not free from limitations. In comparison to other contemporary reports, the sample size in our study was smaller. This was due to the fact that a great deal of effort was required to explain the concept of the study to people and then convince them to participate. Moreover, patients in this study were recruited through a hospital and according to Hossain et al. the number of hospital confirmed cases were low because of the high expense of tests and scarcity of diagnostic facilities. The Directorate General of Health Services (DGHS, the health services regulatory authority in Bangladesh) actively discouraged suspected patients from laboratory diagnosis. The rationales of the DGHS behind this was that significant proportion of people in risk were of the low-income group of the society and that the fatality rate of this infection is extremely low.

With no licensed vaccines and drugs against the virus itself, the disease can only be mitigated by controlling the Aedes vectors. Bangladesh, in that regard, is at risks as mosquito-borne diseases are relatively common. In order to prevent future epidemics, robust surveillance and mosquito-control systems need to be implemented. At the same time, research should be directed towards the genome of the Chikungunya virus to monitor its mutations and possible effects.

Declarations

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Competing Interests

None of the authors have any conflict of interest to declare.

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