

Original Research Article

Risk Factors for Concurrent Malaria and Arbovirus Infections in Handeni, Northeastern Tanzania

ABSTRACT

Aim: We aimed to understand risk factors associated with seropositivity to chikungunya and dengue infections in an area endemic for malaria in a cross sectional analytical study.

Study Design: A structured questionnaire was used for interview to study risk factors for CHIKV and DEN exposure whereas serology (ELISA) for anti-Chikungunya and Dengue virus immunoglobulin M detection was used to determine exposure to the arboviruses. Microscopy was used for malaria parasites detection to understand the extent of interaction between arboviruses and malaria.

Results: A total of 448 participants were screened for anti- chikungunya/dengue IgM and malaria. A seroprevalence of 15.6% and 0.2% was found for Chikungunya and Dengue respectively. Age, less than 16 years ($p=0.011$) and malaria positivity ($p=0.001$) were found to be significant risk factors for Chikungunya IgM seropositivity. Female gender, not keeping livestock, using bed net and travelling outside the study area were associated with reduced risk for CHIKV seropositivity by 28%, 24%, 29% and 37%, respectively. Not using a bed net was twice as much more likely to be malaria-Chikungunya IgM positive compared to bed net use ($OR=2.19$; $p=0.198$).

Conclusion: There is ongoing, transmission of arboviruses in the study region. Chikungunya virus and its co-occurrence with malaria was found to be common in the study population but not Dengue virus. Surveillance of arbovirus transmission in malaria endemic areas should be strengthened to be able to predict fatal outbreaks before they occur.

Keywords: Chikungunya, Dengue, malaria, seroprevalence, risk factors

1. INTRODUCTION

Chikungunya and Dengue viruses are important public health problems that have recently been producing extensive epidemics in tropical urban and semi urban areas nearly worldwide (1;2). Chikungunya disease has been identified in nearly 40 countries worldwide but is known to be endemic in Africa and Asia where it has caused massive and sustained outbreaks, infecting more than 2 million people with an attack rate as high as 68% in some areas (3-5). Dengue infection is currently endemic in more than 100 countries worldwide and the leading cause of illness and death in tropics and sub- tropics . More than 40% of the world's population is at risk from dengue and about 50–100 million dengue infections are estimated to occur worldwide every year (2;4). Despite scarce surveillance data, Chikungunya virus infection has been found to be an important but unrecognized cause of febrile illness in northern Tanzania (6). Recent Dengue epidemic in Dar es Salaam (7) with increased travel and human activities could have promoted more permissive environment for vector-host interaction and spread of dengue infection to other areas.

Little is known on the epidemiology and risk factors for CHIKV and DENV and their interaction with other febrile diseases such as malaria in Tanzania where patients with acute CHIKV and DENV infection are usually misdiagnosed and treated with anti-malaria/antibiotics (6;8). This could lead to a number of consequences including economic loss, selection of resistant strains and increased morbidity and mortality. The aim of this study was to determine seroprevalence and risk factors for chikungunya and dengue viruses and their co-infection with malaria in an area with moderate malaria transmission.

2. METHODOLOGY

2.1 Study Site

This study was carried out in Bondo area in Handeni district within Tanga region in Northern Tanzania. Tanga is an area of moderate to high perennial malaria transmission with malaria prevalence of 13.9% [10]. The study area marks the border between Handeni and Korogwe districts. The study site is 6 kilometers from the main high way to Dar es Salaam and Kilimanjaro and has a total population of 6000 people majority being children under 18 years.

2.2 Study design

A cross-sectional study was conducted from May 2013 to August 2014, enrolling children and adults aged between 2 and 70 years who were residents in the study area. Households were selected randomly from which participants were enrolled only after verification of subject eligibility and obtaining written consent from adult participants or parents/guardians for the case of children. Participants with severe disease were excluded from the study. A structured questionnaire was used to interview adult participants and parents/guardians for the case of children. Information on social demographic characteristics was recorded. Other information recorded included house hold characteristics, the use of insecticide treated bed nets, livestock ownership, history of travel outside the study area for the last three months and the type of mosquito breeding sites around home. Anti chikungunya/dengue virus IgM positive sample was defined as the one with optical density (OD) values $\geq$ the cut-off value in the ELISA reading, (The cut-off value was calculated by adding 0.300 to the mean absorbance of the negative control) and malaria infection was defined as parasite positive results from blood slide smear microscopic reading.

2.3 Sample collection and examination

About 0.5ml of finger-prick blood was collected in Eppendorf tubes with EDTA (ethylenediaminetetraacetic acid) tubes after the site being cleaned with 70% alcohol and allowed to air dry. Thick and thin smears were prepared and stained with Giemsa stain 5% for 30 minutes. Blood slides were examined independently by two experienced laboratory technicians using a light microscope. Discrepant findings were reviewed by a third technician and consensus on positivity was reached. The remaining plasma samples were stored at -20°C for later use in screening for CHIKV and DENV immunoglobulin M antibodies. Anti-Chikungunya/dengue IgM was detected by using Enzyme-Linked Immuno-Sorbent Assay (ELISA) commercial kits (SD chikungunya IgM and dengue IgM Capture ELISA kits - Standard Diagnostics, Inc. Kyonggi-do, Korea).

2.4 Data analysis

Data were entered into a database using excel and exported to Statistical Package for Social Sciences (SPSS) 20.0 software (IBM-SPSS Inc., Chicago, IL, USA) for cleaning. Statistical analysis was done by use of STATA version 12.1. Logistic regression was used to identify risk factors for seropositivity of all infections, individually and collectively. Odds ratios (ORs) and their confidence intervals (95% CIs) were calculated to determine the strength of association between outcome (seropositivity) and individual and household variables. A p value of < 0.05 was considered the cutoff for statistical significance.

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Socio-demographic characteristics

A total of 448 participants were enrolled and interviewed, among which 249 (55.6%) were females and 199 (44.4%) were males. In terms of age categories, 130 (29.0%) were between the age of 2-5 years, 169 (37.7%) were between 6-15 years and 149 (33.3%) were between 16-70 years. The study population was almost evenly distributed in terms of gender and age categories (Table 1).

3.1.2 Risk factors for Chikungunya-Dengue-Malaria co-occurrence

Majority of participants, 352 out of 435 (80.9%) were sharing a bed (>1 persons per bed), 321 of 442 (72.6%) had long grasses around their houses and more than half, 229/447 (51.1%) were not using bed nets at all. A good number of participants, 93/443 (21.0%) were livestock owners (Table 2).

3.1.3 Prevalence of outcomes

Out of 448 participants, 70 (15.6%) were CHIKV IgM positive, 1(0.2%) was DENV IgM positive, 37(8.2%) were malaria positive by microscopy, 13(2.9%) were positive for both CHIKV IgM + malaria and 1(0.2%) was positive for both CHIKV +DENV IgM. There was no malaria+ DENV IgM co-occurrence detected (Table 3).

3.1.4 Risk factors for Chikungunya/Dengue IgM seropositivity

Age and malaria positivity were found to be the risk factors for Chikungunya virus IgM seropositivity by logistic regression. Participants ≥ 16 years old had 62% less risk to become positive for Chikungunya IgM (CHIKV infected) compared to younger participants (OR=0.38; P=0.011). This implies that being less than 16 years old had more risk of being seropositive for anti-CHIKV IgM. Participants who were malaria negative were 70% less likely to be anti-Chikungunya IgM seropositive compared to malaria positive individuals (OR=0.30; P=0.001). With this finding, the inference is that, having no malaria reduced the likelihood of being positive for CHIKV IgM by 70% compared to being malaria positive (Table 4).

The study also showed that, gender (OR=0.72; P=0.22), keeping livestock (OR=0.76; P=0.38), bed net use (OR=1.29; P=0.34), sleeping >1 per bed (OR=1.81; P= 0.14) and traveling outside the study area (OR=1.63; P= 0.16) were not risk factors for Chikungunya virus IgM seropositivity (Table 4). However, despite the absence of statistical evidence, this study has observed that participants sharing a bed (>1 person per bed) had a higher risk for CHIKV IgM positivity by almost 2 times compared to those sleeping alone. Likewise, being female, not keeping livestock, using bed net and travelling outside the study area were associated with reduced risk for CHIKV IgM seropositivity by 28%, 24%, 29% and 37%, respectively (Table 4).

Risk factors for DENV IgM seropositivity and co-occurrence with CHIKV IgM were not analyzed due to the small number of IgM positive samples; i.e 1(0.2%) for both DENV IgM positive and its co-occurrence with CHIKV IgM.

Table 1. Demographic characteristics of participants (n= 448)

Characteristic	Group	Number (n)	*%
Gender	Male	199	44.4
	Female	249	55.6
Age group	2-5years	130	29.0
	6-15 years	169	37.7
	16-70 years	149	33.3

*Percentage was calculated as $n/448 \times 100$

Table 2. Descriptive statistics of risk factors for Chikungunya/Dengue IgM seropositivity and malaria

Risk Factor	Number	%
Number per bed		
Single	83	19.1
2 or more	352	80.9
Livestock ownership		
Yes	93	21.0
No	350	79.0
Bed net use		
Yes	218	48.8
No	229	51.2
Breeding sites		
Uncovered containers	114	25.8
Used car tires	6	1.4

Stream/river/well nearby	1	0.2
Long grasses around house	321	72.6

Numbers indicate the counts and percentage for each risk factor.

Table 3. IgM Seroprevalence of Chikungunya and dengue viruses and their co-occurrence with malaria

Test	Results	Number (n)	%**
CHIKV IgM M	Positive	70	15.6
	Negative	378	84.4
DENV IgM	Positive	1	0.2
	Negative	447	99.8
CHIKV IgM+ Malaria*	Positive	13	2.9
	Negative	435	97.1
CHIKV + DENV IgM	Positive	1	0.2
	Negative	447	99.8

CHIKV means Chikungunya virus, DENV means Dengue virus. *Positivity for asexual forms of pf parasites by microscopy. ** Percentage was calculated as $n/448 \times 100$

126 **Table 4. Risk factors for anti-chikungunya IgM antibodies positivity**

Risk Factor	OR	95% CI	p
Age			
2-5 years	1.00	[1.00,1.00]	Reference
6-15 years	1.03	[0.57,1.85]	0.923
16-70 years	0.38	[0.18,0.80]	0.011*
Number per bed			
1 per bed	1.00	[1.00,1.00]	Reference
>1 per bed	1.81	[0.83,3.96]	0.137
Gender			
Males	1.00	[1.00,1.00]	Reference
Females	0.72	[0.43,1.21]	0.218
Malaria			
Positive	1.00	[1.00,1.00]	Reference
Negative	0.30	[0.14,0.62]	0.001*
Livestock ownership			
Yes	1.00	[1.00,1.00]	Reference
No	0.76	[0.42,1.39]	0.379
Bed net use			
Yes	1.00	[1.00,1.00]	Reference
No	1.29	[0.77,2.16]	0.340
Travel outside study area			
Yes	1.00	[1.00,1.00]	Reference
No	1.63	[0.82,3.23]	0.164

127 OR= Odds Ratios (crude), CI= Confidence Interval, *p= p value; p≤0.05 is significant

128 Reference= the value on which the degree of risk estimation was based.

129

130 **3.2 DISCUSSION**

131 The current study was designed to assess the prevalence of IgM antibodies against CHIKV and
 132 DENV in a malaria endemic area and assess the extent of co-infections between these viruses and
 133 malaria and their associated risk factors, in order to get baseline epidemiological information on
 134 DENV, CHIKV and malaria co-occurrence in our settings. Analysis of serological data and individual
 135 history suggest that at least 70 out of the sampled 148 individuals in our sample (15.6%) had recent
 136 exposure to the CHIKV. In addition, only 1 participant (0.2%) was seen to have recent infection with
 137 DENV. Epidemiological data on DENV and CHIKV transmission in Tanzania has just recently been
 138 reported mostly from large towns and cities in the Southern City of Mbeya (9) and Northern Tanzanian
 139 town of Moshi (6) and more recently the Tanzanian Capital, Dar es Salaam (7). So far, arboviral
 140 infections have received little epidemiological, clinical, and laboratory research not only in Tanzania
 141 but also the sub Saharan region. Partly, this could be due to the chronic scarcity of diagnostic

infrastructure for arboviruses but also the mere absence of solid evidence of their emergency in this region.

Many previous entomological and serological surveys on malaria have been conducted but *Aedes aegypti*, the main vector for CHIKV and DENV has not been reported in entomological surveys in the study area. These surveys, however, were focused primarily on malaria vectors, the *Anopheles*, without prioritizing probable breeding sites for *Aedes*. Out of 148 participants, 37 (8.2%) were positive for asexual forms of malaria parasites. Out of these, 13 were co-infected with CHIKV implying that 13 malaria positives were part of the 70 CHIKV IgM positives.

If the 13 malaria positive cases out of 70 CHIKV IgM positives would have developed clinical symptoms, in the absence of a precise means to distinguish the infections, the remaining 57/70 (81.4%) CHIKV positives, but malaria negative, would very likely be mistaken for malaria. Mixed infections with many etiologic agents that result to misdiagnoses of malaria are not uncommon in malaria endemic areas (10). Despite scanty data, arboviral and malaria co-occurrence should not be under estimated in areas where the environment favors the survival of mosquitoes, regardless of species, since most species would share most ecological and climatic environments suitable as habitats.

The host response to viral infection represents a complex orchestration of divergent pathways designed to eradicate the virus and benefit the host. However, many pathways that are involved in antiviral defense can also have untoward effects on the host, including NK cell and CTL hyper-responses, cytokine hyper responses (11), and severe apoptosis. Thus, the most effective defense response needs to be sufficiently lethal to rapidly kill invading pathogens which remain largely ill-characterized in CHIKV infections. Previously, high levels of CHIKV IgM were reported from a cohort of Indian patients with post-CHIKV rheumatoid arthritis (RA)-like illnesses (12).

Younger age and malaria positivity were found to be risk factors for CHIKV IgM antibody seropositivity in the study population. Previous exposure in the elderly could mean build up of immunity to CHIKV infection as compared to the younger participants. Malaria negative individuals were less likely to be CHIKV IgM positive. One reason could be due to the fact that the two infections are both transmitted by mosquitoes, and that the environment that favors malaria vectors may also favor CHIKV vectors and that malaria is known to alter body immunity which may increase the risk for other infections (13;14). To our knowledge, this is the first study that has revealed the effect of malaria positivity on CHIKV IgM seropositivity in Tanzania.

Moreover, females were found to have less risk from CHIKV IgM positivity compared to males. In our settings, males are usually more active in agricultural activities and they may be more exposed during the vectors peak activity hours, which could increase the chances for mosquito bites. Previously, male gender was found a risk factor for CHIKV seropositivity among adults ≥ 15 years of age in Mayotte, Indian Ocean (15).

Current study findings have also shown that the individuals who were sleeping ≥ 2 per bed had about two times risk of CHIKV IgM positivity compared to those sleeping single. Bed sharing may be indicator for poverty or family low economic status that has also been associated with CHIKV infection elsewhere (15). This may increase the feeding opportunities for mosquitoes, thus maintain their population and anthroponotic transmission.

Analysis on the effect of various risk factors on malaria-chikungunya co-occurrence revealed that non bed net users had more than 2 times risk of being positive for both malaria and CHIKV IgM compared to bed net users. Children for example, are the most affected group with malaria and have higher risk from CHIKV infection. Although malaria is shown by our analyses to be a risk factor for CHIKV positivity, this could also imply overlapping epidemiological factors that lead to their co-occurrence in children. Such factors could be because both diseases are transmitted by mosquitoes whose breeding habitats could be similar and the weak immunological status in children.

It has been documented that *Aedes aegypti* mosquitoes feed indoors and during daytime. However, they are known to have their peak activity hours early in the morning and late in the evening [4]. Children, usually get up late in the morning but go to bed early in the evening, and they also

frequently sleep in day time. These time points when children are in bed coincide with peak mosquito feeding times and thus sleeping unprotected exposes children to mosquito bites.

Malaria and arbovirus co-infections have been reported in malaria endemic areas, mostly with DENV, though in Nigeria a co-infection of 2.8%, 6.3% and 7.4% was reported between malaria and West Nile Virus, DENV and CHIK respectively [17]. However, in this population malaria and CHIKV IgM co-occurrence was 13(2.9%), while there was no observed co-occurrence between malaria and DENV IgM. This prevalence might be lower than the ones reported elsewhere suggesting the possible low population density of DENV infected vectors along with malaria decline by > 50% in Tanzania (16-18).

4. CONCLUSION

There is ongoing, transmission of arboviruses in the study region. Chikungunya virus and its co-occurrence with malaria is common in the study population but not Dengue virus. Surveillance of arbovirus transmission in malaria endemic areas should be strengthened to prevent outbreaks and diagnosis dilemmas.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Approval for this study was obtained from Kilimanjaro Christian Medical University College Research and Ethics Review Committee and permission to conduct the study was authorized by District Medical Officer of Handeni district. All participants and/or parents/guardians were informed on the objectives, procedures and benefits of the study and assured on anonymity and confidentiality. Written consent was obtained from all participants and children's parents or guardians.

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