A cross sectional serologic and epidemiological study of dengue virus infection in north central area of Trinidad and Tobago.

ABSTRACT

Aims: This study was carried out to determine the observed serological and significant epidemiological risk factors for dengue fever infection in a cross-section of the population in Trinidad and Tobago.

Study design: This was a prospective cross sectional study.

Place and Duration of Study: The study was carried out in the department of Paraclinical Sciences of the University of the West Indies, St. Augustine Campus, Trinidad and Tobago, over a period of 10 months, October 2016 to July 2017.

Materials and Methods: Over 450 individuals from a cross section of the population residing in the northern part of Trinidad Island were surveyed. These included individuals suspected of having dengue fever that presented to the health care facilities with complaints of fever along with some other additional symptoms of viral illness. There was no age, gender or ethnic bias. Standardized questionnaire was used to obtain epidemiological data. Blood samples taken from consented participants were analyzed using rapid immune chromatographic tests (ICTs) – Panbio, SD Bioline and Enzyme Linked Immunosorbent Assays (ELISA). The samples were also tested for baseline blood parameters; platelets and haemoglobulin. The epidemiological data was analyzed using SPSS version 21.

Results: Analysis of 380 individuals who fulfilled study criteria revealed that there were no demographic characteristics (age, gender, locality, etc.) that showed statistical significance with having a dengue infection. Retro-orbital pain, headaches and respiratory symptoms (e.g., cough, cold) showed differences that were significant with those having a dengue infection. No statistical significance was found in any comorbidity (diabetes, hypertension and asthma) factors considered and patients with dengue infections. Evaluation of platelet counts showed that only 5.4% samples had abnormal range and while four out of that five tested positive, this was not significant. Monitoring of platelet levels is still very important, but it showed that it is not an indicator of worsening dengue because 95.3% of the positive cases were within normal levels.

Conclusions: Except for nonspecific symptoms observed among patients suspected of dengue fever, there were no other significant factors that were exclusive in identifying dengue infection among the subjects studied. Platelet monitor cannot be used alone as a parameter to determine deteriorating dengue patients.

1. INTRODUCTION

Dengue is now a global public health problem that is endemic in more than 100 countries with an estimated 50 – 100 million infections annually[1, 2.] Dengue fever is an acute manifestation of an arthropod borne viral infection with dengue virus belonging to the *Flaviviridae* family and is transmitted by the bite of a female *Aedes aegypti* mosquito. Four serotypes of the virus are known to exist DEN-1-4[3], and a recently documented fifth serotype appears to have emerged[4]. Classic dengue fever is usually self-limiting, especially in children. Dengue infection manifests in several severe forms, including dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue haemorrhagic fever is associated with re-infection, characterized by the defects in homeostasis and plasma leakage into interstitial spaces associated with increased levels of vasoactive cytokines[5]. This leads to life threatening shock (DSS) in some cases.

The severe syndromes occur in patients with passively acquired or pre-existing, non-neutralizing, heterologous antibody caused by a previous infection with a different serotype of the virus[6]. The

antibodies from the previous infection bind to the new infecting serotype and facilitate viral entry via Fc-receptor binding cells, so the number of antigen-presenting cells is increased at secondary infection[5]. In 2016, there was a recorded 1,801 probable cases alone in Trinidad and Tobago out of the total 9,993 probable cases in the non-Latin (English, French and Dutch) Caribbean[7]. This is a significant decrease in the number of reported cases when compared to 2014; with 9,970 probable cases. As was noted in a prospective sero-epidemiological study, many dengue infections do not produce symptoms and the number of reported cases underestimates the actual prevalence of dengue in the population[8, 9].

The aim of this study was to serologically confirm the frequency of dengue virus infection and determine epidemiological risk factors associated with dengue infections among patients suspected of having dengue fever and attending health care facilities in the north central region of Trinidad and Tobago.

2. MATERIAL AND METHODS

2.1 Study design, sites and population

This was an observational cross sectional study conducted during the period of October 2016 – July 2017, among patients with suspected dengue infection. The study was carried out at two health care facilities of the North Central Regional Health Authority (NCRHA) in Trinidad of the twin Island, Trinidad and Tobago with catchment areas as indicated in the figure below (Fig 1). This area has a high population density in the country and most dengue cases in the past were localized to this region [10], hence the choice as the study area.

This study was carried out among patients who presented to these health care facilities with suspected dengue infection. The study enlisted voluntary participants who gave written consent. Standardized data collection form was used to obtain epidemiological information from the participants. All study participants were seen and physically examined by a medical personnel involved in the study. Suspected dengue infection is characterized by fever along with the following clinical features: anorexia, rash, aches and pains, vomiting and nausea, abdominal pains and warning signs include positive tourniquet test, leukopenia, thrombocytopenia (platelet count $<150 \times 10^9$/L), abdominal tenderness, clinical evidence of plasma leakage and/or increase in haematocrit[11].
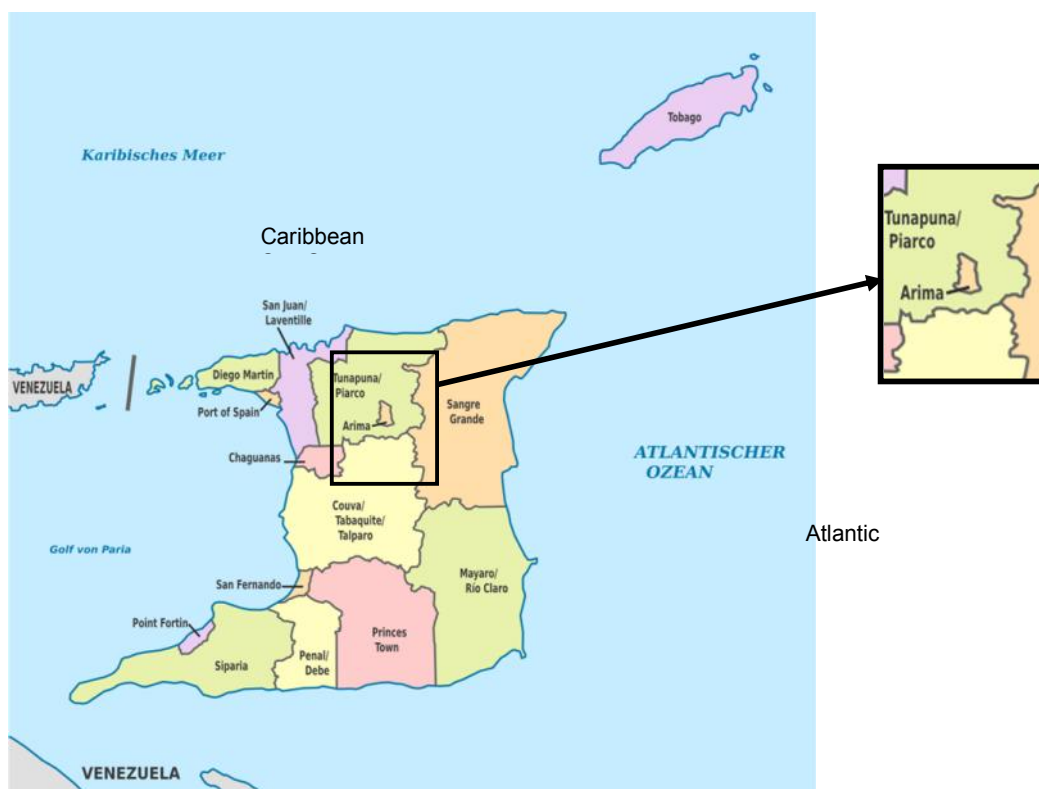
Figure 1. Geographical map of Trinidad and Tobago showing the locality of individuals surveyed for dengue virus fever in Trinidad and Tobago, 2016 – 2017

2.2 Inclusion criteria

All patients of all age groups, gender, ethnic groups, social and educational level who presented to these health facilities with suspected dengue infection symptoms as enumerated above and gave written consent or assent were included in the study. Any patient who did not meet the previously mentioned requirements for suspected dengue infection or did not give consent was excluded from the study.

2.3 Collection of Specimen

A questionnaire was used to obtain patient biodata or information and clinical history. This was administered by one of the trained investigators to avoid bias and misinterpretation or misrepresentation of the responses from the participants.
About 10ml of blood (5ml each in red and purple top tubes) was obtained through venipuncture and transported to the Department of Paraclinical Sciences, The University of West Indies, St. Augustine Campus; and Pathology Laboratory at the Eric Williams Medical Sciences Complex for further analysis. The blood samples were allowed to clot at room temperature, centrifuged and separated as soon as possible. They were then stored at 2-8°C for a maximum of two days or stored frozen at -30°C until complete testing.

2.4 Laboratory Analysis - Complete Blood Count

All samples were subjected to a routine complete blood count as part of the routine services offered to the patients by the health care facilities including platelet counts for each patient.

2.5 Rapid Immuno-chromatographic tests (ICTs)

The samples collected in the red top tubes were subjected to serological analysis using enzyme linked immunosorbent assay - ELISA, (Dengue Virus IgM/IgG capture DxSelect ELISA, Focus Diagnostics, Cypress, PA, USA) for detection of human serum IgM and IgG antibodies in dengue virus (DV) infections. Rapid immune-chromatographic tests (ICT) kits were used for detection of IgM

98   and IgG antibodies, and non-structural protein 1 (NS1) antigen; of sera collected and the results were
99   recorded.
100
101   **2.6 Quality Controls**
102   Controls for both the IgM/IgG ELISA kits were provided as follows: detectable controls (human sera),
103   non-detectable controls (human sera) and cut-off calibrators (human sera). Samples that were
104   collected from asymptomatic and healthy individuals during the time of the study were used as
105   controls for both of the rapid ICT tests. Controls were run every time when procedures were carried
106   out.
107
108   **2.7 Statistical Analysis**
109   Microsoft Excel was used for data entry and data analysis was performed using Statistical Package
110   for the Social Sciences (SPSS) 23.0 software. Chi-square test and Fisher's exact test were used to
111   compare categorical variables. The Chi-square was chosen for determination of association between
112   a tested variable and a positive dengue result. If a relationship existed between any of the variables,
113   the Chi-square value (p value) would reflect the strength of the association. The Fisher's exact test is
114   used in place of the Chi-square to measure the same association for smaller sample sizes. In cases
115   where the frequency counts are fewer than five in a two by two table, the test statistics (p) used is the
116   Fisher's exact value. A probability value (p) of < 0.05 was considered statistically significant.
117
118   **3. RESULTS AND DISCUSSION**
119
120   Over 450 individuals were recruited for the study. Only 380 of these gave consent, completed the
121   questionnaire, got evaluated and gave blood samples and hence were included in the final analysis.
122   Patients included were noted to have come from different ethnic groups of people living in this part of
123   the country as depicted on Figure 1 above. As reported by these patients (Table 1), 38.7% were of
124   mixed ethnicity followed by patients of African descents or ethnicity, 36.6%. The East Indian and
125   Spanish descents groups completed the ethnicity analysis with 22.6% and 1.1 % respectively. Most of
126   the samples were obtained from females (61.3 %) and the median age of all analyzed individuals in
127   the study was 26 years (range, 3 years to 87 years) but the prevalent age group surveyed was
128   between 21 – 30 years (Figure 2). The median time between onset of illness and collection of
129   specimens was 3 days (range, 1 to 50 days).
130
131   The laboratory tests or characterization of the blood samples using the ELISA reference for dengue
132   IgM and IgG, initially classified the analysis as 92.5% positive for dengue and 7.5% non-dengue. Of
133   those that tested positive for dengue, females were in the majority (60.5%) and 32.6% of all positive
134   cases were between the ages of 21-30 years old. Based on the clinical history, presentation of the
135   fever, body aches and headache, the blood samples and the subjects were further defined or
136   classified as acute cases or phase (74.2 %), convalescent cases or phase (18.3 %); and based on
137   immune status, as primary 5.4 % or secondary, 87.1 %.
138   Demographics were the first parameters used to determine what would qualify as risk factors in
139   acquiring a dengue infection. Being of a particular ethnic group had no bearing or significance on
140   whether the patient tested dengue positive. The majority of the positives (35.5%) were found to be of
141   'mixed' descent, followed by African descent (34.4%). There was also no association between living in
142   a particular area and contracting dengue, although most recruits were from the Arima area (Figure 1
143   above) as there was a high percent that tested positive (47.3 %) there.
144   The statistical analysis in this study revealed that retro-orbital pain, respiratory symptoms (cold,
145   cough, runny/stuffy nose) and headache (Table 1) had significant association with samples that tested
146   positive for dengue ($p < 0.05$). More than half (53.3 %) of patients surveyed that tested positive for
147   dengue reported experiencing retro-orbital pain; 88.4 % of dengue- positive patients experienced
148   headaches while 80.2 % experienced respiratory symptoms.
149
150   Platelet levels of the patients were analyzed and categorized as abnormal ($\leq 150 \times 10^9$/L) and normal
151   ($\geq 150 \times 10^9$/L). The largest numbers of dengue positives were found in the age group 21-30, 27.9%
152   in the normal platelet range and 4.7% in the abnormal platelet range (Table 2), however, this
153   difference was not statistically significant ($p = 0.172$). The age group 11-20 (Table 2) showed the
154   second highest number of dengue positives with 18.6%. The mean age of those that tested positive
155   was 29 years old, while the mean platelet counts were 130,000 and 293,000 within the abnormal and
156   normal range, respectively. Except for the age groups 21 – 30 that recorded abnormal platelet counts,
157   all the other age groups had no abnormal platelet counts.

The objective of this study was to use serological analysis to confirm frequency of dengue virus infection and make association between epidemiological risk factors that may exist among the patients suspected of the infection in a cross section of individuals in Trinidad and Tobago. Results from studies such as this can assist physicians stop speculating when it comes to a diagnosis of dengue in our locality as far too many cases go unnoticed or recorded as acute viral illness (AVIs). While accurate laboratory diagnosis can be very helpful in confirming the disease, it will also provide key data on the epidemiology and health burden of dengue, which is very useful for accurate public health surveillance[12]. In this study, similar number of individuals reported their ethnicity to be either of African descent or mixed race; and many of these tested positive for dengue virus infections. Also majority of the participants surveyed gave their location to be Arima area which was also noted to be a significant factor in this study. The high number of positive results in each of these categories appears to only reflect the majority within the sampled population.

Symptoms were statistically analyzed to determine their associations with a dengue virus infection although dengue infections may initially be asymptomatic in 50 – 90% of individuals[13]. The significant ones include retro-orbital pain (eye pain), headaches and respiratory symptoms which are similar to a previous report[14]. Eye pain is particularly common in dengue infection along with headaches but the degree to which they are experienced are not quantifiable and so they remain non-specific. Most patients who tested positive for dengue antibodies also complained of body pains but this was not found to be significant. Reporting of having a previous infection of either dengue, chikungunya or zika, also did not show any differences for those who tested positive. Among the several patients that had already suffered from a dengue infection, none of them showed signs or symptoms that were more severe than those who said they never were infected with dengue. As dengue is one of the most under reported tropical disease[3], it is very possible that patients who claimed to have never had dengue may be unaware of the past diagnoses seeing that symptoms are non-specific and home remedies are administered by patients themselves until symptoms subside. This way, there is and can be no accurate monitoring of the actual disease or possible burden of infection.

Figure 2. Age and gender distribution of 380 patients surveyed for dengue virus infections in Trinidad and Tobago, 2016 - 2017.
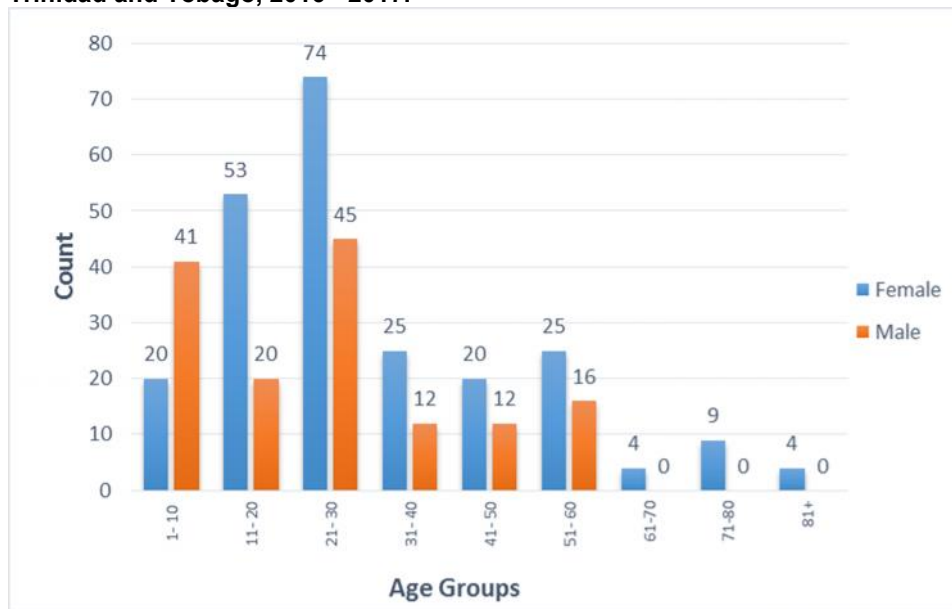
Table 1. Characteristic features of 380 patients surveyed for dengue virus infection in the north central regional health authority, Trinidad and Tobago, 2016 – 2017 (%).

Characteristics		Negative	Positive	p value	
analyzed		28 (7.7)	352 (92.3)		Number
Demographics	Male	8 (28.6)	139 (39.5)	0.702	
	Female	20 (71.4)	213 (60.5)	0.702	
	African descent	12 (42.9)	131 (37.2)	1.000	
	East Indian descent	4 (14.3)	82 (23.3)	1.000	
	Mixed	12 (42.9)	135 (38.4)	1.000	
	Spanish	0	4 (1.2)	1.000	
Symptoms	Rash	4 (14.3)	41 (11.6)	1.000	
	Headache	16 (57.1)	311 (88.4)	0.054*	
	Retro-orbital pain	0	188 (53.5)	0.012*	
	Body pain	20 (71.4)	274 (77.9)	0.654	
	Joint pain	4 (14.3)	176 (50)	0.115	
	Diarrhoea	8 (28.6)	119 (33.7)	1.000	
	Cough, cold, runny nose	8 (28.6)	282 (80.2)	0.007*	
	Gum/Nose bleeds	0	33 (9.3) 1.000		
Previous infections	None	28 (100)	254 (72.1)	0.184	
	Dengue	0	65 (18.6)	0.600	
	Chikungunya	0	29 (8.1) 1.000		
	Zika	0	4 (1.2)	1.000	
Co-morbidities	Hypertension	0	17 (4.7)	1.000	
	Diabetes	0	8 (2.3)	1.000	
	Diabetes + HTN	0	4 (1.2)	1.000	
	Asthma	0	37 (10.5)	1.000	
	Other – Arthritis, PCOS, etc.	4 (14.3)	29 (8.1)	0.479	
	None	24 (85.7)	254 (72.1)	0.670	
Mosquito Conditions					
	Many mosquitoes in the area	24 (85.7)	237 (67.4)	0.428	
	Nets/Screens at home	0	61 (17.4)	0.593	
	Blocked drains around house	0	70 (19.8)	0.342	
	Get bitten often	20 (71.4)	193 (54.7)	0.459	
	No mosquito problems	4 (14.3)	111 (31.4)	0.670	

*p < 0.05 is considered statistically significant. p values were determined using Chi – square tests. Data are presented as n (%) or median (interquartile range); HTN – hypertension, PCOS – polycystic ovary syndrome

Table 2. Age distribution of enzyme linked immunosorbent assay (ELISA) and platelet counts of patients tested for dengue virus infection in a cross section of Trinidadian patient, 2016-2017 (%).

Age Groups	Negative ELISA		Positive ELISA	
	Abnormal	Normal	Abnormal	Normal*
1 – 10	0 (0)	16(4.3)	0(0)	45(11.8)
11 – 20	0(0)	8(2.1)	0(.0)	65(17.2)
21 – 30	4(1.0)	0(.0)	16(4.3)	98(25.8)
31 – 40	0(.0)	0(.0)	0(.0)	38(9.7)
41 - 50	0(.0)	0(.0)	0(.0)	33(8.6)
51 – 60	0(.0)	0(.0)	0(.0)	41(10.8)
61 – 70	0(.0)	0(.0)	0(.0)	4(1.1)
71 – 80	0(.0)	0(.0)	0(.0)	8(2.2)

| 258 | 81+ | 0(.0) | 0(.0) | 0(.0) | 4(1.1) |
| 259 | TOTAL | 4(1.0) | 24(6.4) | 16(4.3) | 336(88.3) |

260 --

261 The Platelet counts were considered as normal (≥150,000) and abnormal (≤150,000)

264 Co-morbidities such as hypertension, diabetes mellitus and asthma are among the non-
265 communicable illnesses that are most prevalent in Trinidad and Tobago[15]. If left unmanaged they can
266 lead to high morbidity and mortality rates. Whether or not either of these had any effects on the
267 prevalence of dengue infection was also investigated. Most of those that were found positive for
268 dengue infection showed no significant associations with having any medical conditions (asthma,
269 diabetes, hypertension), being on any particular medications or having received any vaccines in the
270 last two months prior to being enrolled. However, a study in Asia, attempted to show the association
271 of diabetes mellitus with DHF. The study found that female, Chinese, age group 30-49 years with pre-
272 existing diabetes mellitus or diabetes with hypertension were risk factors of developing DHF during an
273 epidemic while dengue serotype 2 was predominant[16]. Neither of these characteristics were found to
274 show any significant differences in our current study despite age group (21-30 years), gender (more
275 females than males) or ethnicity (more of mixed ethnic group descents) gave more numbers; and also
276 the fact that 25.5% of the sampled population in this study suffered from comorbidities.

278 In our locality where we do not have problem of distinguishing dengue from malaria that produces low
279 platelet counts[17], hence platelet counts have been one of the most important factors in tracking the
280 progress of dengue infection. Monitoring platelet levels however, should not be the sole criteria to
281 presume dengue infection as many patients in this study tested dengue positive without abnormal
282 platelet counts that is indicative of plasma leakage. In a study by Lovera et al, they investigated
283 platelet count as a risk factor of shock. Using a cut-off of $< 100 \times 10^9$/L they found that children who
284 did not develop shock exhibited similar percentage level of thrombocytopenia compared to patients
285 who eventually developed it (47 % vs 49 %). The results were similar when the comparison included
286 patients only with platelet counts < 50,000/mL (28 % vs 25.6 %)[18]. In this present study, the mean
287 platelet count for positive samples in patients 1- 10 years of age was 295×10^9/L. Those with
288 abnormal counts were only found in the 21- 30-year-old age group and 80% of them tested positive
289 for dengue virus. This adds up to 4.3% of those who tested positive but was not of any significance.
290 None of the patients had platelet levels that were $< 50 \times 10^9$/L.

292 The Pan American Health Organization (PAHO) has already issued a release of the number of
293 reported cases of dengue and severe dengue in the Americas by country for epidemiological week 39
294 (updated October 13, 2017). After week 32 in Trinidad and Tobago the number of probable reported
295 cases were 206, none of which were laboratory confirmed[7]. This may very well be an indication of
296 how the health sector had prioritized dengue infection in this country. It is no longer important to
297 identify or confirm a true case of dengue as long as we successfully manage its viral symptoms. It is
298 of utmost importance that all probable cases not only be reported but confirmed, especially if
299 headway is to be made on curbing infection and development/implementation of a vaccine. The WHO
300 has stated their position on the newly developed vaccine (CYD-TDV) saying that countries should
301 consider introduction of the dengue vaccine only in geographic settings where epidemiological data
302 indicate a high burden of disease[19]. The vaccine, also known as Dengvaxia, is a live attenuated
303 (recombinant) tetravalent vaccine that was created to be administered by 3 injections of 0.5ml given at
304 6-month intervals. We cannot indicate high burden of disease if the epidemiological data being
305 collected is recorded incorrectly or disregarded. Hence, all assumptions for diagnoses need to be
306 confirmed and confirmed by the most accurate methods.

308 4. CONCLUSION

310 Despite the limitations of this study that include the small sample size and lack of use of molecular
311 tests for confirmation of dengue virus, the study still detected positive cases of dengue virus infections
312 in the country. Except for nonspecific symptoms observed among patients suspected of dengue fever,
313 there were no other significant factors that were exclusive in identifying dengue infection among the
314 subjects studied. Platelet monitor cannot be used alone as a parameter to determine deteriorating
315 dengue patients.

316

CONSENT

Informed consent was also obtained from each of the patients, along with assent from children that were included in the study. Patients under the age of 18 were considered as children.

ETHICAL APPROVAL

Ethics approval for this study was obtained from the Campus Ethics Committee of the University of the West Indies St. Augustine Campus and the North Central Regional Health Authority (NCRHA) Ethics Committees. The study was carried out in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

REFERENCES

1. Guzman A, Isturiz RE. Update on the global spread of Dengue. Int J Antimicrobial Agents 2010; Suppl 1:S40-S42.
2. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL et al. The global distribution and burden of dengue. Nature 2013; 496:504-7
3. Gubler DJ. Dengue and dengue hemorrhagic fever. Lin Microbiol Rev 1998; 11:480-496.
4. Mustafa MS, Rasotgi V, Jain S, Gupta V. Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. Med J Armed Forces India 2015; 71(1), 67-70. doi:10.1016/j.mjafi.2014.09.011
5. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis 2000; 181(1), 2-9. doi:10.1086/315215
6. Brooks GF, Carroll KC, Butel JS, Morse SA. Jawetz, Melnick & Adelberg's Medical Microbiology (26 ed.) 2013; New York McGraw Hill Lange
7. PAHO. (2017). Number of Reported Cases of Dengue and Severe Dengue (SD) in the Americas, by Country. Figures for 2016 (to week noted by each country). Epidemiological Week / EW 52 (Updated February 6, 2017). Accessed online June 10, 2018 from www.paho.org/hq/dmdocuments/2016/2016-cha-dengue-cases-jan-26-ew-52.pdf
8. Chadee DD, Shivnauth B, Rawlins SC, Chen AA. Climate, mosquito indices and the epidemiology of dengue fever in Trinidad (2002–2004). Annals of Tropical Medicine & Parasitology 2007; 101(1), 69-77. doi:10.1179/136485907X157059
9. Campbell CA, George A, Salas RA, Williams SA, Doon R, Chadee DD. Seroprevalence of dengue in Trinidad using rapid test kits: a cord blood survey. Acta Trop 2007; 101(2), 153-158. doi:10.1016/j.actatropica.2006.11.009
10. Chadee DD, Williams FL, Kitron UD. Impact of vector control on a dengue fever outbreak in Trinidad, West Indies, in 1998. Trop Med Int Health 2005; 10(8), 748-754. doi:10.1111/j.1365-3156.2005.01449.
11. WHO. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control: New Edition 2009; World Health Organization. Accessed online June 10, 2018 from www.who.int/rpc/guidelines/9789241547871/en
12. Parkash O, Shueb RH. Diagnosis of Dengue Infection Using Conventional and Biosensor Based Techniques. Viruses 2015; 7(10), 5410-5427. doi:10.3390/v7102877
13. Kyle JL, Harris E. Global spread and persistence of dengue. *Anual Rev Microbiol* 2008; 62:71-92.
14. Gregory J, Santiago LM, Arguello DF, Hunsperger E, Tomashek KM. Clinical and laboratory features that differentiate dengue from other febrile illness in an endemic area – Peurto Rico, 2007 – 2008. Am J Trop Med Hyg 2010; 82:922-929
15. WHO. Non communicable Diseases (NCD) Country Profiles, 2014: Trinidad and Tobago. Accessed online June 10, 2018 from www.who.int/nmh/publications/ncd-profiles-2014/en
16. Pang J, Salim A, Lee VJ, Hibberd ML, Chia KS, Leo YS, Lye DC. Diabetes with hypertension as risk factors for adult dengue hemorrhagic fever in a predominantly dengue serotype 2 epidemic: a case control study. PloS Negl Trop Dis 2012; 6(5), e1641. doi:10.1371/journal.pntd.0001641
17. Chadwick D, Arch B, Wilder-Smith A, Panton N. Distinguishing dengue fever from other infections on the basis of simple clinical and laboratory features: application of logistic regression analysis. J Clin Virology 2006; 35:147-153
18. Lovera D, Martinez de Cuellar C, Araya S, Amarilla S, Gonzalez N, Aguiar C, Arbo A. Clinical Characteristics and Risk Factors of Dengue Shock Syndrome in Children. Pediatr Infect Dis J 2016; 35(12), 1294-1299. doi:10.1097/INF.0000000000001308

377   19. WHO. Dengue vaccine: WHO position paper Weekly Epidemiological Report 2016; 30(91), 349-
378   364.