Original Research Article

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.

Keywords: Dengue fever, ELISA, Epidemiology, Serology, Panbio, Trinidad and Tobago.

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.

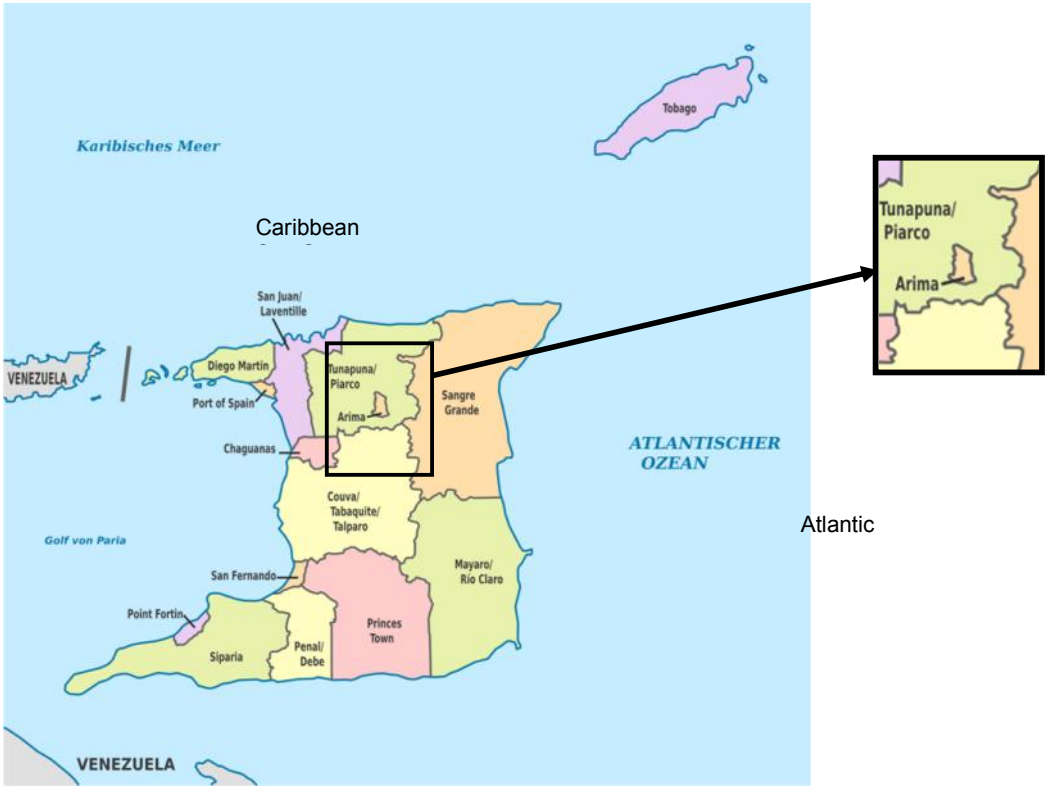
2. MATERIAL AND METHODS

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

and IgG antibodies, and non-structural protein 1 (NS1) antigen; of sera collected and the results were recorded.

2.6 Quality Controls

Controls for both the IgM/IgG ELISA kits were provided as follows: detectable controls (human sera), non-detectable controls (human sera) and cut-off calibrators (human sera). Samples that were collected from asymptomatic and healthy individuals during the time of the study were used as controls for both of the rapid ICT tests. Controls were run every time when procedures were carried out.

2.7 Statistical Analysis

Microsoft Excel was used for data entry and data analysis was performed using Statistical Package for the Social Sciences (SPSS) 23.0 software. Chi-square test and Fisher's exact test were used to compare categorical variables. The Chi-square was chosen for determination of association between a tested variable and a positive dengue result. If a relationship existed between any of the variables, the Chi-square value (p value) would reflect the strength of the association. The Fisher's exact test is used in place of the Chi-square to measure the same association for smaller sample sizes. In cases where the frequency counts are fewer than five in a two by two table, the test statistics (p) used is the Fisher's exact value. A probability value (p) of < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

Over 450 individuals were recruited for the study. Only 380 of these gave consent, completed the questionnaire, got evaluated and gave blood samples and hence were included in the final analysis. Patients included were noted to have come from different ethnic groups of people living in this part of the country as depicted on Figure 1 above. As reported by these patients (Table 1), 38.7% were of mixed ethnicity followed by patients of African descents or ethnicity, 36.6%. The East Indian and Spanish descents groups completed the ethnicity analysis with 22.6% and 1.1 % respectively. Most of the samples were obtained from females (61.3 %) and the median age of all analyzed individuals in the study was 26 years (range, 3 years to 87 years) but the prevalent age group surveyed was between 21 – 30 years (Figure 2). The median time between onset of illness and collection of specimens was 3 days (range, 1 to 50 days).

The laboratory tests or characterization of the blood samples using the ELISA reference for dengue IgM and IgG, initially classified the analysis as 92.5% positive for dengue and 7.5% non-dengue. Of those that tested positive for dengue, females were in the majority (60.5%) and 32.6% of all positive cases were between the ages of 21-30 years old. Based on the clinical history, presentation of the fever, body aches and headache, the blood samples and the subjects were further defined or classified as acute cases or phase (74.2 %), convalescent cases or phase (18.3 %); and based on immune status, as primary 5.4 % or secondary, 87.1 %.

Demographics were the first parameters used to determine what would qualify as risk factors in acquiring a dengue infection. Being of a particular ethnic group had no bearing or significance on whether the patient tested dengue positive. The majority of the positives (35.5%) were found to be of 'mixed' descent, followed by African descent (34.4%). There was also no association between living in a particular area and contracting dengue, although most recruits were from the Arima area (Figure 1 above) as there was a high percent that tested positive (47.3 %) there.

The statistical analysis in this study revealed that retro-orbital pain, respiratory symptoms (cold, cough, runny/stuffy nose) and headache (Table 1) had significant association with samples that tested positive for dengue ($p < 0.05$). More than half (53.3 %) of patients surveyed that tested positive for dengue reported experiencing retro-orbital pain; 88.4 % of dengue- positive patients experienced headaches while 80.2 % experienced respiratory symptoms.

Platelet levels of the patients were analyzed and categorized as abnormal ($\leq 150 \times 10^9$/L) and normal ($\geq 150 \times 10^9$/L). The largest numbers of dengue positives were found in the age group 21-30, 27.9% in the normal platelet range and 4.7% in the abnormal platelet range (Table 2), however, this difference was not statistically significant ($p = 0.172$). The age group 11-20 (Table 2) showed the second highest number of dengue positives with 18.6%. The mean age of those that tested positive was 29 years old, while the mean platelet counts were 130,000 and 293,000 within the abnormal and normal range, respectively. Except for the age groups 21 – 30 that recorded abnormal platelet counts, 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.

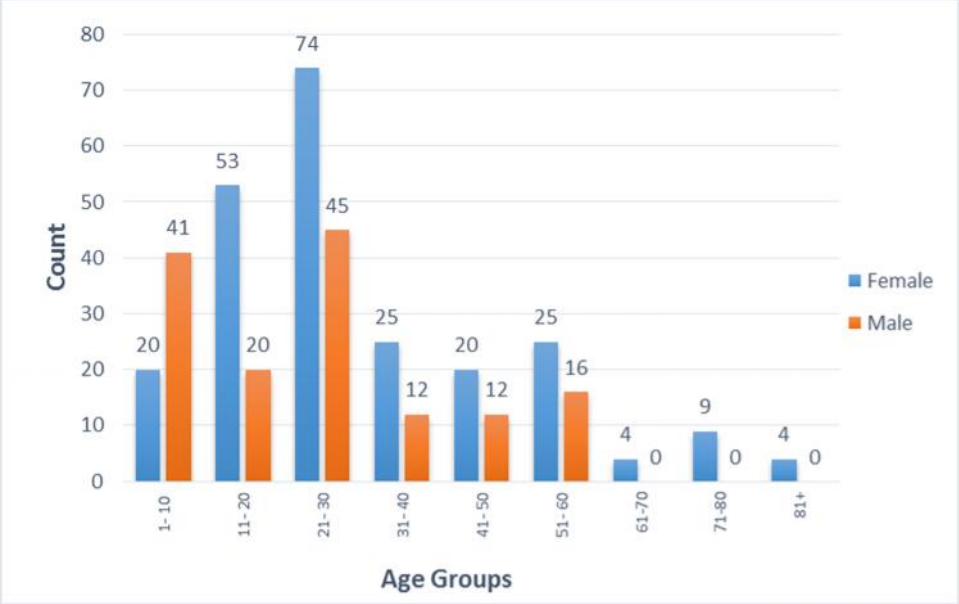


Table 1. Characteristic features of 380 patients surveyed for dengue virus infection in the north central regional health authority, Trinidad and Tobago, 2016 – 2017 (%).

Characteristics			Negative	Positive	*p* value
Number analyzed			28 (7.7)	352 (92.3)	
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)

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

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

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

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

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

4. CONCLUSION

Despite the limitations of this study that include the small sample size and lack of use of molecular tests for confirmation of dengue virus, the study still detected positive cases of dengue virus infections in the country. 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.

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.

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