

Predictive Diagnostic Value of the Tourniquet Test and Correlation of Laboratory Profile for the Diagnosis of Dengue Infection Among Patients <18 Years Old Admitted at San Lazaro Hospital from September 2015 to November 2015

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Introduction: Tourniquet Test has been widely used locally and internationally as the cheapest, fastest and most efficient way of diagnosing dengue infection. This is so efficient that the World Health Organization has been active in promoting this as a diagnostic tool for tropical countries like the Philippines.

Objective: To determine the accuracy of the tourniquet test in the diagnosis of dengue infection among patients aged <18 years old admitted at San Lazaro Hospital from September to November 2015

Methodology: The retrospective research design was utilized. These variables were included: the socio-demographic and clinical variables as to the patients' age, gender, body mass index and presence of co-morbid conditions, clinical manifestations (duration of fever, headache, abdominal pain, rashes and bleeding episodes, hemoglobin level, WBC, Platelets, NS1, IgG, IgM and Hematocrit values). Once these were noted, the researcher coded the results. Coding was done in Microsoft Excel.

Results: Patients were mostly in the 12 to 17 years age group, males (54.1%), in the normal weight category (88.1%) and had no co-morbid conditions (77.1%). Clinical manifestations of dengue included fever of ≥ 4 days (56%); abdominal pain (61.5%); and gum bleeding (56.4%). As regards laboratory profile of patients, low hemoglobin levels were observed (77.5%); low WBC count (78.9%); low platelet counts (100%); positive for NS1 (71.1%); positive for Immunoglobulin G (82.6%) and Immunoglobulin M (85.3%). There was a 65% accuracy of cases when tourniquet test was performed among patients with dengue. There was a strong correlation between dengue infection and WBC ($r = 0.915$; $p = 0.028$); platelets ($r = 0.663$; $p = 0.003$); hematocrit ($r = 0.557$; $p < 0.01$); Non-Structural Protein 1 ($r = .753$; $p = .031$); IgG ($r = 0.566$; $p = 0.002$); IgM ($r = 0.510$; $p = 0.009$) and Tourniquet test ($r = 0.513$; $p = 0.045$). No significant correlation was observed for cases of dengue infection and hemoglobin ($r = 0.026$; $p = 0.702$) and platelets ($r = 0.026$; $p = 0.702$).

Conclusions: The dengue infection was found mostly to be in the 12 to 17 years of age; predominantly males. BMI was not found to be significant factor for dengue infection since most cases were in the normal weight category. Clinical presentations of dengue infection commonly observed in this research were fever with a duration of 4 days and above, headache, abdominal pain and gum bleeding. Majority had abnormal levels of hemoglobin, low WBC, and low platelet count. Biomarkers for dengue infection noted in the study were positive NS1, presence of IgG and IgM. Tourniquet test yielded 65% accuracy if the number of petechiae cases is considered. This could indicate that as an initial tool for diagnostics, presence of it could be considered for dengue infection. Strong correlation with tourniquet test, and laboratory parameters like WBC, platelets, hematocrit, NS1, IgG and IgM were found which are indicative that these variables have strong association to possible infection of dengue. Any abnormalities found within these laboratory parameters must be considered for dengue evaluation.

Key words: Dengue Fever, Tourniquet

INTRODUCTION

Dengue is prevalent in many tropical and sub tropical regions. The clinical diagnosis of dengue is still complex. Dengue is transmitted by the bite of a mosquito infected with one of the four dengue virus serotypes. It is a febrile illness that affects infants, young children and adults with symptoms appearing 3-14 days after the infective bite. Dengue is not transmitted directly from person to person and symptoms range from mild fever, to incapacitating high fever, with severe headache, muscle and joint pain, and rash. There is no vaccine or any specific medicine to treat dengue. People who have dengue fever should rest, drink plenty of fluids and reduce the fever using paracetamol.

Severe dengue (also known as Dengue Hemorrhagic Fever) is characterized by fever, abdominal pain, persistent vomiting and bleeding and is a potentially lethal complication, affecting mainly children. Early clinical diagnosis and careful clinical management by trained physicians and nurses increase survival of patients. Infection with dengue virus is one of the leading causes of illness and hospital admission in Southeast Asian children.¹⁻² Infection may be asymptomatic, or may result in a variety of clinical syndromes ranging from dengue fever (DF), a non-specific febrile illness, to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).

The main feature differentiating DHF from DF is an increase in vascular permeability resulting in leakage of plasma from the intravascular compartment to the extravascular space.³⁻⁴ In severe DHF, the loss of fluid is critical and hypovolemic shock develops resulting in potentially lethal DSS. Thrombocytopenia, coagulation abnormalities and clinical bleeding of varying severity are features of DHF and DSS, but may also occur in DF.⁵⁻⁶ In 1997, the World Health Organization (WHO) listed the tourniquet test (TT) as a criterion for dengue hemorrhagic fever, and that the positive test reflects both capillary fragility and thrombocytopenia.⁷ Studies suggested that the test has a greater positivity rate in individuals with more severe forms of the disease but cannot exclude dengue infection.⁸⁻⁹ Evaluation of the utility of tourniquet Test

to diagnose or exclude dengue has been proposed in previous studies,¹⁰⁻¹¹ with mixed results.

In a tropical country like the Philippines, Dengue carrying mosquitoes are considered to be very prevalent. In this regard, dengue infection is considered to be very prevalent. There is a widespread infection occurring not only in the adult population but also to children in the country. This is so efficient that the World Health Organization has been active in promoting this as a diagnostic tool for tropical countries like the Philippines.¹² In this line, the researcher considers the following to benefit from the results of this undertaking.

This study was conducted to determine the accuracy of the admission tourniquet test in the diagnosis of Dengue infection among patient ages <18 years old admitted at San Lazaro Hospital for the period of September to November 2015.

MATERIALS AND METHODS

Research Methodology

In this study, the researchers utilized the retrospective research design. The data were analyzed in relation to the outcome of the research. The main aim of this research study was to determine the accuracy of the admission tourniquet test in the diagnosis of dengue infection among patient ages <18 years old admitted at the San Lazaro Hospital from September to November 2015.

Done at the San Lazaro Hospital, the study population was composed of patients residing in Manila and nearby cities and municipalities. Patients having clinical manifestations of dengue infection as well as abnormal laboratory tests were considered to have dengue infection.

The confidence level used for statistical tests in this study was 95%. The design effect for random sample was set at 1.0 with an estimated 218 cases as sample size. These data were recorded and analyzed. Since chart review was done in this study, informed consent was not obtained.

Inclusion and Exclusion Criteria

Specifically, cases of dengue infection noted from September to November 2015 were included. Patients 18 years of age and below with clinical manifestations of dengue infection and admitted at pavillions 3, 4, 7 and 8 of San Lazaro Hospital were included. The following were excluded: cases from 19 years of age and those confirmed to be not dengue infection based on the laboratory results, patients admitted in other areas of the hospital and cases with missing data.

Data Collection Procedure

Coordination with the authorities of the Department of Family Medicine was done first. Suggestions were solicited and integrated into the protocol in order to improve the research content. The researchers waited for the approval before data collection.

The researchers collected data on the patients' socio-demographic and clinical profiles including patients' age, gender, body mass index and presence of comorbid conditions. The laboratory profiles (hemoglobin level, WBC, platelets, NS1, IgG, IgM and hematocrit) and clinical manifestations of dengue fever (duration of fever, headache, abdominal pain and rashes) were noted and coded. Coding was done for nominal variables. For interval and ratio variables, the researchers noted the figures.

Statistical Treatment of Data

The researcher initially recorded all the results in Microsoft Excel. Coding was done in this program. Appropriate statistical analyses were utilized in this study. The following data processing tools were used:

a. Frequency Distribution and Percentage. These were used to determine the socio-demographic profile of dengue infected patients admitted at the San Lazaro Hospital according to age, gender, BMI and presence of co-morbid conditions. This was also done to determine the clinical

presentations such as days of fever, headache, abdominal pain and rashes. They were also used to determine the laboratory profile of dengue infected patients such as hemoglobin level, WBC, platelets, NS1, IgG, IgM and hematocrit. Lastly, this was done to determine the type of dengue infection based on the results of tourniquet test of patients admitted at San Lazaro Hospital.

b. Pearson Correlation of Coefficient. This was done to determine the correlation between the type of dengue infection and the results of tourniquet test of patients admitted at San Lazaro Hospital

c. t-test for Independent Samples. This was used to determine the significant difference between the type of dengue infection based on the results of tourniquet test when grouped according to laboratory profile

Ethical Considerations

Coding of patients' data was used instead of utilizing the names and to insure confidentiality of information. The researchers declared no conflict of interest to conduct this study.

RESULTS AND DISCUSSION

Most cases (105) were in the 12 to 17 years of age. This constituted the 48.2% of the population. Of these cases, 98 (45%) were in the dengue with warning signs and only 7 cases were in the severe dengue category. This comprised the 3.2% of the population. Conversely, the least in rank were the 17 cases (7.8%) under the 2 to 6 years of age group. All of these 17 (100%) cases were in the dengue with warning signs category.

The data showed that dengue infection was common in this pediatric age group. In the study by Gubler, it was disclosed that dengue infection was more common in the pediatric age group. In this particular undertaking, children from 7 to 15 years of age were mostly infected with dengue compared to those who were in the early

30 to 40 years of age, also similar to the findings of the present study where those infected were 12 to 17 years of age.¹³ Another research also found it most common in the 13 to 17 years age group.¹⁴ This potentially explains why dengue infection commonly affects those in the pediatric age group.

In this analysis, 118 (54.1%) males were found to be infected with dengue. Of the 118 cases, 109 (50%) had dengue with warning signs while only 9 (4.1%) had severe dengue. Among the females, 100 cases (45.9%) were noted. Of the 100 cases, 94 (43.1%) had dengue with warning signs while 6 (2.8%) had severe dengue.

The results showed that males are more likely to be infected with dengue. Nair, et al. found in their study that females were more prone to develop dengue than the male group.¹⁵ They never argued though that gender has something to do with infection of dengue, Kahmat and Rajit, et al. had different findings in which more males contacted the infection.¹⁶ Chakravarati, et al. had the same results wherein more males were found to be more infected.

Most participants in this study (192) were in the normal weight category. Of the 192 cases, 178 (81.7%) had dengue with warning signs while only 14 (6.4%) were in the severe dengue classification. The least in rank were the 9 cases (4.1%) considered to be obese. All cases had dengue with warning signs.

The data signified that dengue infection could happen to any patient regardless of his weight classification. Similar results were noted in the study by Mao and Zhang in which cases of infection occurred across varying weight groups. This means that cases of dengue were equally distributed across varying weight classifications or body mass indices.¹⁷ De Mattos, et al. explored cases of dengue in Brazil and this yielded similar results as far as body mass index of patients is concerned. In their investigation, it was found that dengue infection was commonly observed among the pediatric age group. Children with normal weight could acquire the disease. This was also observed among those who were in the underweight and obese categories. The authors inferred that dengue

infection does occur in people who seem to be healthy and could happen to those who were in their normal weight as well.¹⁸

Of the 168 cases, 156 (71.6%) had dengue with warning signs while only 12 (5.5%) had severe form of dengue. Conversely, the least in rank were the 11 cases of diabetes as co-morbid condition. The highest number under this category had dengue with warning signs. This comprised 5% of the population while only 1 (5%) had severe form of dengue.

One hundred twenty two (56%) had fever ≥ 4 days. Of these 122 cases, 115 (52.8%) had dengue with warning signs. Only 7 cases (3.2%) were severe. Ninety six (44%) had fever for 1 to 3 days.

The results of the study were similar to those of Akram, et al. which explored the duration of fever among infected individuals. In their study, most patients had fever of at least 4 days to 9 days prior to diagnosis.¹⁹ The investigation carried out by Khan, et al. showed a dengue fever mean duration of 5 to 7 days.²⁰

Headache was noted in 122 cases (56%) and 111 (50.9%) were in the dengue with warning signs category. Only 11 cases (5%) were in the severe dengue category. The results could indicate headache as a clinical manifestation of dengue. Cobelens, et al. stressed that headache was observed among those infected with dengue. As fever accompanied headache, the authors attributed this clinical manifestation to the onset of fever.²¹

One hundred thirty four (134) cases were reported to have abdominal pain. This comprised the 61.5% of the population. Of the 134 cases, 128 (58.7%) were patients with dengue with warning signs while 6 (2.8%) had severe dengue. Only 84 (38.5%) did not have abdominal pain. Studies indicated abdominal pain as an early warning sign of dengue infection as observed by Kamath and Ranjit in their study.²²

Of the 123 cases in the study, 113 (51.8%) were found to be in cases of dengue with warning signs while 10 cases (4.6%) were in the severe form of dengue. Overall, 59 cases (27.1%) had no bleeding. On the other hand, 36 cases (16.5%) had nose bleeding. Of the 36 cases, 34

(15.6%) were in the dengue with warning signs while 2 (.9%) were in the severe form of dengue.

Bleeding is a classic symptom of dengue infection. In most cases, gum bleeding is observed. Hemorrhage happens due to the very low percentage of platelets in the blood. This clinical manifestation should be managed well to avoid medical complications.²³ According to Malik, et al., bleeding occurs in the late stage of dengue infection and should be given immediate medical attention.²⁴

In this analysis, 130 cases (59.6%) had no rashes at all. Only 88 cases (40.4%) had rashes. Of the 88 cases, 84 (38.5%) were in the dengue with warning signs while 4 (1.8%) were in the severe form of dengue.

There were 169 cases (77.5%) in this category. Of the 169 cases, 156 (71.6%) were in the dengue with warning signs and 13 cases (6%) were in the severe dengue case. Conversely, ranked least were 49 cases (22.5%) with normal hemoglobin level in the blood.

Low hemoglobin level was also observed in some studies. Thomas, et al. found low hemoglobin levels among cases of dengue in the pediatric population.²⁵ Saleh, et al. also observed low hemoglobin levels in the blood of patients suffering from dengue infection.²⁶

A total of 172 (78.9%) cases had low WBC. Of the 172 cases, 162 (74.3%) were found to be in dengue with warning signs and only 10 (4.6%) were in the severe form of dengue. Conversely, ranked least were the 46 cases (21.1%) with normal WBC.

As regards data on WBC, a dramatic decrease of WBC among cases of dengue infection was noted in this study. The results of this present study were almost similar with those obtained from the research of Ali, et al. which found that the level of WBC for cases of dengue in the general population was low.²⁷

In this study, all cases (218) had a very low number of platelets.

As the general hallmark of infection, dengue virus generally reduces the platelet count of individuals suffering from this infection. Hence, bleeding could be observed in severe cases due to their low platelet counts.²⁸

A large number of cases (155) were positive for NS1. In this category, 143 (65.6%) were in the dengue with warning signs while only 12 (5.5%) were in the severe dengue classification. Ranked least were the 63 cases (28.9%) which tested negative for NS1.

The results were generally expected by the researchers since NS1 is a confirmatory test for dengue infection. Chakravarti, et al. have discussed in their study that a positive test for non-structural protein antigen is a biomarker intended to detect the presence of infection among dengue cases. Hence, it is expected that most cases would test positive for such analysis.²⁹

There were 180 cases (82.6%) in this category and in which 169 (77.5%) were in the dengue with warning signs. Only 11 (5%) were in the severe dengue category. Only 38 cases (17.4%) tested negative for IgG.

In this study, 186 cases (85.3%) tested positive for this. Of the 186 cases, 173 (79.4%) were in dengue with warning signs. Only 13 (6%) were in the severe form of dengue. The least in rank were 32 cases (14.7%) who tested negative for IgM.

IgG and IgM together with NS1 are biomarkers normally utilized to detect the presence of dengue infection. A combination of serological studies involving non-structural protein1 antigen (NS1Ag), IgM and IgG antibodies were used for diagnosis. If the presentation was early (1-5 days), rapid diagnostic kits for NS1Ag alone were used in cases where clinical suspicion of dengue-like illness was there. In all other cases, NS1Ag, IgM and IgGAb were done by rapid diagnostic kits to confirm dengue infection. Use of the 2012 WHO Classification into dengue ± warning signs and severe dengue has been suggested by WHO for triage of patients during an epidemic. The presence of NS1 antigen positivity, IgM positivity or IgG seroconversion by rapid tests in a clinical setting consistent with dengue fever was treated as positive.³⁰

One hundred ten (50.5%) cases had normal hematocrit levels. Of the 110 cases, 107 (49.1%) were found to be with dengue with warning signs and 3 (1.4%) were in the severe dengue category. There were 108 cases (49.5%) with high hematocrit levels. Of these, 96 (44%)

were in the dengue with warning signs while 12 (5.5%) were severe dengue.

It could be seen that the number of petechiae reached > 20. A very high number of 142 cases (65.1%) were noted in this study. Of the 142 cases, 132 (60.6%) were noted to have dengue with warning signs while 10 (4.6%) were in the severe form of dengue. All cases included in this study tested positive for dengue based on the laboratory analysis such as the NS1, IgG and IgM. However, there were cases with ≤ 19 petechiae which still tested positive for dengue infection. In this study, only 65% were cases of dengue infection when the tourniquet data were considered. This indicates that tourniquet test as a diagnostic tool could not provide accurate information with regard to the dengue infection among patients.

It must also be considered that the TT can be influenced by disease progression, age, gender,³¹ circulating strains, and virus circulation patterns over a determined period. This test can also yield a positive result for other pathological conditions with varying indexes, such as with typhoid fever and Japanese encephalitis,³² although the TT has lower sensitivity for these diseases than for dengue. In this study, 68.5 % of patients who had dengue confirmed by serological diagnosis showed a positive TT result. Understanding this condition could prevent infections with clinical presentations similar to dengue from being misdiagnosed and thus provide a better chance for accurate treatment in adequate time. Such evidence shows the complexity involved in the clinical diagnosis and management of dengue. In 1997, the World Health Organization (WHO) listed the tourniquet test (TT) as a criterion for dengue hemorrhagic fever, and that the positive test reflects both capillary fragility and thrombocytopenia. Studies suggested that the test has a greater positivity rate in individuals with more severe forms of the disease but does not exclude dengue infection.³³

Table 1 shows the correlation between dengue infection, tourniquet test and laboratory profile of dengue patients. It could be seen that there is a strong correlation between dengue infection and WBC ($r .915$; $p .028 < .05$);

platelets ($r .663$; $p .003 < .05$); hematocrit ($r .557$; $p .000 < .05$); non-structural protein 1 ($r .753$; $p .031 < .05$); IgG ($r .566$; $p .002 < .05$); IgM ($r .510$; $p .009 < .05$); and tourniquet test ($r .513$; $p .045 < .05$). On the other hand, no significant correlation was observed for cases of dengue infection and hemoglobin ($r .026$; $p .702 > .05$); platelets ($r .026$; $p .702 > .05$); neutrophils ($r .057$; $p .403 > .05$); lymphocytes ($r .057$; $p .401 > .05$).

Table 1. Correlation between dengue infection and tourniquet test and laboratory parameters for patients admitted at San Lazaro Hospital

Variable	r	strength	p-value	Conclusion
HgB	.026	Negligible	.702	Not Significant
WBC	.915	Strong	.028	Significant
Platelets	.663	Strong	.003	Significant
Neutrophils	.057	Negligible	.403	Not Significant
Lymphocytes	.057	Negligible	.401	Not Significant
Hematocrit	.557	Strong	.000	Significant
NS1	.753	Strong	.031	Significant
IgG	.566	Strong	.002	Significant
IgM	.510	Strong	.009	Significant
Tourniquet Test	.513	Strong	.045	Significant

The results of this undertaking suggest that WBC, platelets, hematocrit; non-structural protein 1, IgG, IgM, and data obtained from tourniquet test were very highly correlated with dengue infection. The data obtained in this study signified that abnormalities with all of the aforementioned can be used in diagnosing possible dengue infection. As regards tourniquet test, many researchers have found that presence of 10 petechiae after the tourniquet test could indicate the presence of dengue infection.³⁴⁻³⁵ Further clinical diagnosis is needed to confirm dengue infections and may involve specific measurements of antibody responses³⁶, as these were for years considered to be the diagnostic

standard.³⁷ Therefore, the data obtained in the study only underscored that infection of dengue could be diagnosed accurately using such tests and laboratory data from patients.

CONCLUSIONS

1. The general characteristics of patients with dengue infection were found to be in the 12 to 17 years of age. Though cases of dengue were also noted to be present in the 12 years old and below age category. As regards gender, males dominated the number of cases of dengue infection. The results were in concordance with the data observed in the general population in the local and international settings as well. Body mass index was not found to be a factor for dengue infection since most cases were in the normal weight category.

2. Clinical presentation of dengue infection commonly observed in this research was fever with a duration of 4 days and longer. Headache was reported among the pediatric population observed in this undertaking. Abdominal pain and gum bleeding were observed as well due to the low number of platelets. The results indicated that these clinical manifestations based on the results of the study should be considered for dengue infection when found.

3. As for the laboratory profile of dengue infected patients admitted at San Lazaro Hospital, majority had abnormal levels of hemoglobin. A low WBC was observed among the majority of cases. All cases of dengue infection had a low platelet count. This was expected in the study since a low platelet number corresponds to possible dengue infection. Biomarker for dengue infection noted in the study was NS1. Presence of IgG and IgM was noted in this undertaking as well.

4. Tourniquet test yielded 65% accuracy if the number of petechiae cases is considered. It is important to highlight in this study that petechiae of 19 or less should be considered negative for dengue, however, petechiae presence still yielded dengue infection based

on the laboratory results performed. This could indicate that as an initial diagnostics tool presence of it could be considered for dengue infection.

5. Strong correlations with tourniquet test, and laboratory parameters like WBC, platelets, hematocrit, NS1, IgG and IgM were found. This indicates that these variables have strong association to possible infection of dengue. Any abnormalities found within these laboratory parameters must be considered for dengue evaluation.

RECOMMENDATIONS

1. Strict monitoring of all laboratory parameters of patients suspected of dengue must be done. This ensures that patients are closely monitored well and unwanted complications are prevented.

2. Increasing the number of hospital beds in order to accommodate large number of patients must be done. With this, patients who are in need of medical assistance could be met.

3. Furthering the information campaign of the local government in terms of eradicating dengue carrying mosquitoes should be done

4. If possible, vaccination for dengue must be provided for free especially to those who are at high risk

For the Community

1. Any signs of fever for an extended period of time should be assessed by medical professionals. This is to ensure that any complications brought by lack of medical check up are prevented

2. Regular use of insecticides and cleaning of sewage system are some activities which could dramatically decrease dengue carrying mosquitoes in the area.

3. Every household must use mosquito nets at home to prevent dengue carrying mosquitoes from transmitting the virus to humans

For Future Researches

1. Exploring cases of dengue infection in the adult population as this could help physicians in treating this problem.
2. Cross analysis of results involving laboratory profile and tourniquet data may be considered for further evaluation.

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