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Editorial for 1st issue 2018

Welcome to the 1st issue of The PCMC Journal for 2018!

As you may have noticed, all six articles in this issue are research studies on dengue done by our trainees. Admittedly most of these researches are not very ambitious, with most focusing on the clinical features of local patients with dengue seen in PCMC. But they do reflect the continuing interest and concern for our patients with this very common yet devastating illness. Hopefully this augurs well for more in-depth studies in the future, even as we continue to be a center of care for children with dengue.

On a final note, we are well on track to fulfilling our commitment to publish two issues of the journal a year. This will mean a greater chance of being included in the WPRIM (Western Pacific Region Index Medicus) database so that our articles will be searchable on the internet, and we can have more access to the resources of the Philippine Council for Health Research Development and other government bodies.

Wish us luck!

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Instructions to Authors:

The Philippine Children's Medical Center Journal (PCMC Journal) is a peer-viewed journal that is published bi-annually and publishes original scientific papers in the field of basic and clinical pediatric research. The articles it accepts for publication may be in the form of collective and current reviews, original papers, case reports, lectures, essays, editorials, abstracts or letters to the editor.

All manuscripts, correspondence and editorial business should be sent to PCMC Journal, Office of Research Development, Philippine Children's Medical Center, Quezon Ave., Quezon City. Manuscripts are received with the understanding that they are not under simultaneous consideration by another publisher. Accepted manuscripts become the permanent property of the Journal and may not be republished without permission from the Editor. These manuscripts are subject to editorial modifications to bring them in conformity with the style of the journal. Statements or views expressed by an author or authors are not the responsibilities of the editor or publisher.

Cover Letter

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General Guidelines

1. One original and two duplicate manuscripts should be submitted. An electronic copy must also be submitted on a floppy or compact disc. The manuscript should be typed double-spaced throughout with 1 ¼ cm (½ inch) paragraph indentation, using only one side of each 22x28 cm (8 ½ x 11 inch) opaque bond paper with 3-cm margins (1 ¼ inch) all around. Preferred font styles and sizes are: Times New Roman 12, Arial 11, Tahoma 11, & Verdana 11.

2. The manuscript should be arranged in sequence as follows: (1) Title Page (2) Abstract (3) Text (4) References (5) Tables (6) Figures & Illustrations. A manuscript for an original article should not exceed 25 typewritten pages (including tables, figures, Illustrations and references). The text for case reports should not exceed 10 pages, including the visual aids and references.

3. References should be selective and pertain directly to the work being reported.

4. All the sheets of the manuscript should be labelled with the family name of the main/first author (all in capital letters) and page number (in Arabic Numeral) printed on the upper right corner.

Title Page

1. The title should be as concise as possible. Include only the full names of the authors directly affiliated with the work starting with the first name, middle initial if any, and last name. The highest educational attainment or title of the authors should be included as an attachment whenever appropriates; name and location of no more than three institutional affiliations may be included.

2. If the paper has been presented in a scientific program or convention, provide a footnote giving the name, location and date of the meeting.

Abstract

For original articles, the abstract should contain no more than 200 words and should have a structured format consisting of the objective, methodology, results and conclusion. For case reports, the abstract should be from 50 to 75 words and need not be structured. At least 3 keywords, preferably using terms from the Medical Subject Headings (MeSH) list of Index Medicus, should be listed horizontally under the abstract for cross-indexing of the article.

Text

1. Generally, the text should be organized consecutively as follows: Introduction, Materials and Methods, Results and Discussion (and Conclusion).
2. All References, tables, figures and illustrations should be cited in the text, in numerical order.
3. Even if commonly employed, all abbreviations should be spelled out once, the first time they are mentioned in the text, followed by the abbreviations enclosed in parentheses. Subsequently, the same abbreviations may be used instead of the long names.
4. All measurements and weights should be System International (SI) units.
5. Acknowledgements to individuals/ groups of persons, or institution/s should be included at the end of the text just before the references. Grants and subsidies from government or private institutions should also be acknowledge

References

1. References in the text should be identified by Arabic Numerals in enclosed parentheses on the same line as the preceding sentence.
2. References should be typed double-spaced on separate sheet. They should be numbered consecutively in the order which they are mentioned in the text.
3. All references should provide inclusive page numbers.
4. Journal abbreviations should conform with those used in the Index Medicus.
5. A maximum of three authors per article can be cited; beyond that, “et al.” is added.
6. The style/punctuation conforms to that recommended by the ICMJE, which is the ANSI standard style used by the NLM, and should follow the format of the examples shown below:

Journal Article

Padua FR, Paspe MG. Antinuclear antibody in the rheumatic and non-rheumatic diseases among Filipinos. Acta Med Philipp 1990; 26(2): 81-85.

Book

Huth EJ. How to write and publish papers in the medical sciences. Philadelphia: ISI Press, 1982.

Tables

1. Cite all tables consecutively in the text and number them accordingly. Create tables preferably using a spreadsheet program such as MS Excel with one table per worksheet. Tables should not be saved as image files. The content of tables should include a table number (Arabic) and title in capital letters above the table, and explanatory notes and legends as well as definitions of abbreviations used below. Recommended font is Arial Narrow size 8.

2. Each table must be self-explanatory, being a supplement rather than a duplicate of information in the text. The use of too many tables is discouraged.

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Figures or graphs should be identified by Roman Numeral/s with titles and explanations underneath. The numbers should correspond to the order in which the figures/graphs occur in the text. It is recommended that figures/graphs also be submitted as image files (preferably as .jpeg or.gif files) of high resolution. All identifying data of the subject/s or patient/s under study such as name, case numbers, etc., particularly in case reports should be removed.

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1. All illustrations/photographic prints should be submitted in duplicate and placed in a separate envelope.
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5. The principal author's last name and illustration number should be placed at the back of each illustration/ photo print in soft pencil. An arrow on the gummed label indicating the top should be drawn.
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Contact Information
For comments, questions, and concerns, contact:

Paul Matthew D. Pasco, MD, MSc

Editor – In – Chief

PCMC Journal

Office of Research Development

Philippine Children's Medical Center

Quezon Ave., Quezon City

Tel No. 9240838 or 5889900 local 356

Fax No. 9240840

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THE CLINICAL PROFILE AND OUTCOME OF CHILDREN WITH DENGUE ENCEPHALITIS AT THE PHILIPPINE CHILDREN'S MEDICAL CENTER: A RETROSPECTIVE STUDY FROM JANUARY 2011-JUNE 2017

KRISTINE ALVARADO-DELA CRUZ, MD, MADELYN P. PASCUAL, MD
REMEDIOS O. SALONGA, RN AND MARIA EVA LUNA-DIZON, MD

ABSTRACT

BACKGROUND: Dengue, a mosquito-borne flavivirus infection, is hyperendemic in the Philippines. One of the rare complications is dengue encephalitis, characterized by altered sensorium, elevated liver enzymes, and high dengue-specific antibody titers. The actual incidence of dengue encephalitis is underestimated due to problems of pathogen detection. To address this, an integrated surveillance system has been in place since 2007, which detects laboratory-confirmed dengue meningoencephalitis from blood and CSF samples via IgM capture ELISA.

OBJECTIVES: To describe the clinico-demographic profile and outcome upon hospital discharge of laboratory-confirmed dengue encephalitis patients admitted at the Philippine Children's Medical Center (PCMC) from January 2011 to June 2017.

METHODS: This is a retrospective observational study to describe laboratory-confirmed dengue encephalitis cases aged 0-18 years at PCMC from January 2011 to June 2017.The clinico-demographic profiles and outcomes of patients were collected using chart review, and variables were analyzed using descriptive statistics.

RESULTS: 14 cases of laboratory-confirmed dengue encephalitis children were reviewed. None had known previous dengue infection nor received dengue vaccination. Following nonspecific signs and symptoms, neurological manifestations developed with a median of 2 days, the most common being seizures, most of which were generalized, and decreased sensorium. Most common abnormal EEG waveforms were generalized background slowing; neuroimaging was normal or showed cerebral edema. Full recovery upon discharge was seen in half the patients reviewed, 3 showing partial recovery from neurologic signs and symptoms, and 3 others were discharged with neurologic sequelae. One infant expired.

RECOMMENDATIONS: Prospective studies with larger sample sizes that will follow-up on the patient's long-term outcome are recommended.

KEYWORDS: Dengue, dengue encephalitis, dengue severe, viral encephalitis, acute meningoencephalitis.

INTRODUCTION

Dengue is a major health problem in most tropical and subtropical areas[1] and is the most rapidly spreading mosquito-borne viral disease in the world. In the last 50 years, incidence has increased 30-fold with increasing geographic expansion to new countries and, in the present decade, from urban to rural settings. An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries[2].

The number of dengue cases reported annually to WHO has increased from 0.4 to 1.3 million in the decade 1996–2005, reaching 2.2

million in 2010 and 3.2 million in 2015(6,7).In 2013 dengue was estimated to be responsible for approximately 3.2 million severe cases and 9000 deaths.[3]Severe dengue is a leading cause of serious illness and death among children in some Asian and Latin American countries[3]. Dengue encephalitis is an extremely rare manifestation of severe dengue disease.[12]

In the Philippines, in which dengue is hyperendemic, the incidence of dengue cases seems to show an increasing trend in the recent years. In January 1 to July 16, 2016, the suspected dengue cases reported nationwide is 22.9% higher compared to the same time in 2015.[3]Dengue continues to be a leading cause of morbidity in the Philippines. The disease ranks ninth among the ten leading causes of morbidity[16], with the majority of cases being reported in the 5-14 years age group.[17]

There is no specific cure for the disease, thus efforts have been focused on early detection, optimal management, prevention through vector control and dengue immunization. The Philippine Food and Drug Authority (FDA) licensed a tetravalent, live attenuated dengue vaccine manufactured by Sanofi Pasteur last December 2015 for use in individuals 9 to 45 years of age.[19]

Murthy has classified the spectrum of neurological manifestations seen in dengue into 3 categories: 1)those related to neurotropic effect of the virus, like: encephalitis, meningitis, myositis and myelitis;2) those due to the systemic complications of infection, like: encephalopathy, stroke and hypokalemic paralysis, and 3) finally, post-infectious complications, like: encephalomyelitis, optic neuritis and Guillain Barré syndrome.[11]Many studies have shown dengue infection causing encephalitis with high morbidity and mortality.

Dengue encephalitis patients usually present with altered sensorium, elevated liver enzymes and high antibody titers at the time of admission.[10]Acute encephalitis, defined by the presence of an inflammatory process of the brain in association with clinical evidence of neurologic dysfunction, is a serious and potentially debilitating condition, which may lead to adverse outcomes of prolonged neurologic sequelae or death.

The annual incidence of dengue encephalitis is most likely underestimated, especially in developing countries because of problems with pathogen detection. In the Philippines, the Epidemiology Bureau of the Department of Health established the Philippine Integrated Disease Surveillance and Response (PIDSR) system in 2007, under which the surveillance on Acute Encephalitis Syndrome (AES) and Bacterial Meningitis (BM) falls. An integrated surveillance for Acute Meningitis-Encephalitis Syndrome (AMES) was the established in 2014 as a combination of both AES and BM, which aims to provide this data. The Philippine Children's Medical Center has been a sentinel site since 2011.

An Acute Meningoencephalitis Syndrome (AMES) Surveillance case is a person with sudden onset of fever and at least one of the following: change in mental status (including altered consciousness, confusion or inability to talk), new onset of seizures (excluding simple febrile seizures), neck stiffness and other meningeal signs. Suspected dengue cases that meets the clinical case definition defined by AMES surveillance and a laboratory confirmed dengue infection (as defined by the presence of dengue specific-IgM antibody in serum or CSF detected by dengue NS1 or IgM-capture ELISA, in the absence of co-infection with other etiologic agents) were considered to have dengue encephalitis.

Presently, there are limited local studies describing the clinico-demographic profiles and outcomes of dengue encephalitis cases in the Philippines.

METHODOLOGY

This is retrospective observational study. Hospital charts of children aged 0-18 years admitted at the Philippine Children's Medical Center from January 2011to June 2017, with dengue encephalitis were enrolled in the study. Due to the rarity of cases that were seen in this institution, purposive sampling was done, wherein all cases that fulfilled the inclusion and have none of the exclusion criteria, were enrolled.

All the following criteria were fulfilled prior to study enrolment:

1. Children aged 0-18 years

2. Admitted at the Philippine Children's Medical Center from January 2011 to June 2017.

3. Patients who fulfill the clinical case definition used in AMES surveillance, and are laboratory confirmed cases of acute dengue encephalitis, as defined as a probable dengue case with dengue-specific IgM antibody in a CSF or serum sample detected by Dengue NS1 or IgM-capture ELISA.

Patients who presented with the following were excluded:

1. Cases that turned out to be positive for a bacterial, tuberculous, fungal, parasitic, other viral or immune etiology.

2. Patients with encephalomyelitis (eg. Acute Disseminated Encephalomyelitis)

3. Patients without samples submitted for routine CSF and serum analyses

The Child Neurology admission and referral census from January 2011- June 2017wasreviewed, to look for patients who had been admitted as probable cases of Central Nervous System (CNS) infection and eventually discharged as dengueencephalitis. The Acute Meningoencephalitis Syndrome (AMES) Surveillance reports from January 2011-June 2017were retrieved from the National Reference Laboratory at the Research Institute for Tropical Medicine, to search for laboratory-confirmed cases of dengue encephalitis. Approval was obtained from the Philippine Children's Medical Center (PCMC) Institutional Review Board (IRB).

Hospital charts of patients who fulfilled all of the inclusion and with none of the exclusion criteria were retrieved and reviewed. The following demographic and clinical data were then noted and reviewed in the study-defined patient data sheet: age, gender, geographic location, nutritional status, clinical history, receipt of dengue vaccine, presenting features (fever, headache, vomiting, altered sensorium, seizures), clinical signs (speech disturbance, cranial nerve involvement, motor deficit, cerebellar signs, involuntary movement, meningeal signs, papilledema), duration of hospital stay, co-morbidities, management as well as supporting laboratory and imaging examinations done. Clinical outcome were categorized upon the subject's disposition upon discharge, whether there was full recovery, characterized as complete resolution of previously noted neurologic changes; partial recovery of neurologic changes; presence of neurologic sequelae; or death. An attempt to retrieve and review the outpatient follow-up charts was done, of which none can be located. Some neuroimaging and encephalogram results

done post-hospital discharge were located, however, and were subsequently reviewed.

Descriptive statistics was used to summarize the clinical characteristics of the patients. Frequency and proportion was used for nominal variables, median and range for ordinal variables, and mean and standard deviation for interval/ratio variables. All valid data was included in the analysis. Missing variables was neither replaced nor estimated. STATA 12.0 was used for data analysis.

RESULTS

During the period covered by the study, 14 cases of dengue encephalitis were recorded at our institution, of which 8 were males and 6 were females (Table 1). Most (n=10) were from National Capital Region, while two each were from Regions III and IV-A. The median age at disease onset was 2.5 years (range 3 days to 15 years). There were four neonates, two older infants, three preschool-aged children, and five adolescents. Most were of normal weight, however, four patients represented opposite extremes in the weight spectrum, two being obese and two, were severely wasted (the youngest), and underweight (6 years old) respectively. No child was previously vaccinated against dengue virus or had a prior dengue infection. The febrile period lasted for a median of 6 (range 3—11) days, while hospital stay amounted to a median of 13.5 (range 6—41) days. All patients were referred to the intensive care unit. Of the 14 patients enrolled, 9 patients were managed as severe dengue, 4 as neonatal sepsis with CNS infection and 1 as Viral Encephalitis, unspecified. Aside from fluid support (100%) and antibiotics (71%) given to treat concomitant bacterial infection or healthcare-associated infection, the medical interventions consisted of drugs to raise blood pressure (21%) and therapeutic rehabilitation (7%). Eleven patients had concomitant illnesses that were also addressed during the hospital stay.

Table 1. Demographic and clinical profile of patients with dengue encephalitis (n=14)

	Frequency (%); Median (Range)
Age at onset (years)	2.5 years (3 days to 15 years)
Gender Male Female	 8 (57.14) 6 (42.86)
Region of Location/Residence NCR III IV - A	 10 (71.4) 2 (14.3) 2 (14.3)
Weight (kg)	12.3 (2.1 to 65)
Height (cm) (n = 3)	61 (51 to 156.6)

Table 1. (Continuation)

	Frequency (%); Median (Range)
BMI (kg/m^2) (n=3)	15.59 (15.38 to 21.23)
Nutritional assessment*	
Obese	2 (14.3)
Overweight	0
Normal	10 (71.4)
Wasted / underweight	1 (7.14)
Severely wasted	1 (7.14)
Day of illness upon admission	3 (1 to 6)
Duration of febrile phase (days)	6 (3 to 11)
Hospital stay (days)	13.5 (6 to 41)
Time of onset of fever to development of neurologic changes (days)	2 (1 to 5)
Presence of comorbidities/ concomitant illnesses	8 (57.14)
Previous hospitalization	1 (7.14)
Previous dengue infection	0
Intervention given**	
Intravenous fluid support	14 (100)
Antibiotics	10 (71.4)
Pressors	3 (21.43)
Rehabilitation	1 (7.14)
Received dengue vaccine	0

All patients were admitted during the first week of illness, ranging from the 1st to the 6th day (median 3rd day). Fever was the most common presenting sign among patients (100%). Initial signs and symptoms were nonspecific, which included decreased appetite/poor suck (79%), cough and colds (64%), vomiting (43%), irritability (36%),and headache (21%).Rash was seen in 36% (n=5), loose stools in 29% (n = 4), and abdominal findings in 29% (n = 4). There was gastrointestinal bleeding in two children, as evidenced by hematemesis in one and coffee ground material in the orogastric tube in another. Three patients (21%) became jaundiced, with no evidence of hepatomegaly. Enlargement of the liver was noted in 2 patients (Table 2).

The time that elapsed from the onset of the febrile period of dengue infection until the onset of the neurological changes ranged from 1 to 5 days. More than half (57%) of children developed a decrease in sensorium over their hospital course. Other neurological changes included changes in behavior (29%), disorientation (21%), and incoherent words (14%). The youngest patient exhibited spasticity, nuchal rigidity, and a bulging anterior fontanel. Babinski reflex and hyporeflexia were noted in one 10-year-old patient. Seizures, mostly generalized (n=7), were recorded in 71% of patients. This presenting symptom was the most common reason for hospital admission.

Table 2. Presenting symptoms, clinical signs, neurologic changes and neurologic examination findings seen in patients with dengue encephalitis (n=14)

	Frequency (%)
Presenting symptoms	
Fever	14 (100)
Decreased appetite/poor suck	11 (78.57)
Cough/colds	9 (64.3)
Vomiting	6 (42.9)
Irritability	5 (35.7)
Loose stools	4 (28.6)
Headache	3 (21.4)
Abdominal pain	2 (14.3)
Clinical Signs	
Rash	5 (35.7)
Flushed skin	4 (28.6)
Pallor	3 (21.4)
Jaundice	3 (21.4)
Bleeding	2 (14.3)
Abdominal enlargement	2 (14.3)
Hepatomegaly	2 (14.3)
Neurologic changes	
Seizures Generalized Focal Both	10 (71.4) 7 (70) 1 (10) 2 (20)
Decreased sensorium/ Increased sleeping time	8 (57.14)
Behavioral changes	4 (28.6)
Disorientation	3 (21.4)
Incoherent words	2(14.3)
Aphasia	1 (7.1)

Table 2. (Continuation)

	Frequency (%)
Neurologic examination findings	
Nuchal rigidity	3 (21.4)
Bulging anterior fontanel	2 (14.3)
Spasticity	1 (7.1)
Development of Babinski reflex (abnormal for age)	1 (7.1)
Hyporeflexia (DTR +1)	1 (7.1)

Upon admission, more than half (57%) of children had depressed hemoglobin for age (Table 3), 5 (83%) of which were within normal range for weight based on nutritional assessment at the time of confinement. Only one (7.1%) patient, aged 6 years, developed hemoconcentration, an evidence of plasma leakage due to increased vascular permeability[19].The average peripheral WBC count is 7,500 cu/L. The lowest count was 1,700 cu/L and the highest was 28,400 cu/L. Platelet count ranged from 4/mm^3 to 132/mm^3 before returning to normal values. Majority (92.9%) demonstrated leukopenia and thrombocytopenia.

Among those tested, majority had elevated ALT (8 of 10) and AST (5 of 6). One patient with consistently normal BUN registered high creatinine levels (maximum of 114.92 μmol/L). Hypokalemia was noted in half the children whom serum electrolytes were measured, other results were mostly normal. Three had high glucose, while one had hypoglycemia. Partial prothrombin time was prolonged in 40% of 10 children, and PT INR in 50% of these. Half of the patients showed radiologic evidence of pneumonia, only 3 (21%) showed pleural effusion.

All patients had cerebrospinal fluid (CSF) analysis done, 8 were collected during the first week of illness. On gross examination, 3 patients (21%) had hazy CSF samples (Table 3). Pleocytosis for age was seen in in only one patient. CSF white blood cells (WBC) ranges from 0-8 cells x 10^6/L (median 2.14 cells x 10^6/L), all with 100% lymphocytic predominance. Other findings included slight hypoglycorrhachia (14.3%), and a mild increase in the protein level (14.3%) in 2 patients (43 and 45% respectively). Majority of the patients (71.4%) had normal CSF analysis.

Table 3. CSF findings of patients with dengue encephalitis (n=14)

	Mean ± SD; Median (Range)	Frequency (%)	
		Colorless/ Clear	**Xanthochromic/ Hazy**
Gross exam		11 (78.6)	3 (21.4)
		Normal	**With Pleocytosis**
WBC (cells x 10^6/L)	2.14 ± 2.6	13 (92.9)	1 (7.1)
		Normal	**Elevated**
Protein (g/L)	0.33 (0.2 to 1.07)	12 (85.7)	2 (14.3)
		Normal	**With Hypoglychorrachia**
CSF: SERUM glucose	65.86 ± 17.8	12 (85.7)	2 (14.3)

Table 4. Dengue-specific test results of patients with dengue encephalitis (n=14)

	Frequency (%)	
	Positive	**Negative**
Dengue IgM (+) in CSF	5 (35.7)	9 (64.3)
Dengue IgM (+) in serum	14 (100)	0
Dengue IgG (+) in serum (n=6)	4 (66.7)	2 (33.3)
Dengue NS1 (+) in serum (n=2)	2 (100)	0

All patients had CSF and serum samples submitted for analysis for the presence of dengue IgM via capture enzyme-linked immonosorbent assay (ELISA) (Table 4). All 14 (100%) patientsin the study had acute dengue infection as evidenced by the finding of seropositivity for dengue-specific IgM, but only 5 (36%) had dengue IgM in CSF. Six of the 14 patients had serum Dengue IgG determination, 4 (67%) of which were positive. Three patients were positive for both serum dengue IgG and IgM. Only 2 patients were tested for serum Dengue NS1, both of which were positive. Concomitant blood and CSF bacterial cultures were done on all patients, all of which were negative for any bacterial pathogen.

Neuroimaging revealed intracranial changes in67% of patients, with findings such ascerebral edema (55.6%), and meningeal enhancement (33.3%).

Abnormality in EEG wave forms was seen in 7 (87.5%) of 8 children who underwent EEG. Findings included continuous slowing of the background activity (86%), focal slowing (71%) and epilepticform discharges (29%). Follow up EEG done in three patients, 3 weeks to a month after hospital discharge showed normal results in 2 patients, and significant improvement in the generalized background slowing in one patient. The most commonly used medication for seizure control was phenobarbital (92.9%).

Table 5. Outcome of patients with dengue encephalitis

Outcome	Frequency (%)
Fully recovered from neurologic changes	7 (50)
Partiallyrecovered from neurologic changes	3 (21.43)
Neurologic sequelae present	3 (21.43)
Death	1 (7.14)

The outcomes of patients in terms of full or partial recovery from neurologic signs and symptoms, presence of neurologic deficits, or death was determined upon discharge assessment and available ancillaries. Seven of the 14 patients (50%) have fully recovered from neurologic changes, three (21%) showed partial recovery, and three (21%) had neurologic sequelae upon discharge. One child, an infant, expired (Table 5).

DISCUSSION

Dengue virus belongs to the Flaviviridae family, which includes a number of neurotropic viruses such as Japanese encphalitis virus, St. Louis encephalitis virus, and tick-borne encephalitis virus.[14]Thesigns and symptoms, as well as the characteristic laboratory markers for severe dengue were not seen in most patients with dengue encephalitis. Mufazzar in 2006 supports this finding, as he found that not all patients with dengue encephalitis develop complications of severe dengue.[28]

Antenatal and post-partum dengue infection secondary to vertical transmission has been documented to occur in neonates in several earlier reports [23,24]. Interestingly, this study found four neonates who had dengue specific IgM via serology, three of which also had dengue IgM in the CSF. None of these neonates were suspected to have an acute dengue infection during the hospital admission and were instead treated as cases of neonatal sepsis. Review of the patients' clinical course revealed that all four neonates fulfilled the minimum criteria for probable dengue.CSF analysis was done due to the consideration of concomitant CNS infection, and samples have routinely been sent to AMES surveillance for analysis. Results of the AMES surveillance were not known during the hospital stay of the patients, and all four neonates were discharged. Three neonates fully recovered, the last neonate still showing signs of fair suck, but improving activity upon discharge. Three out of four neonates demonstrated dengue IgM in the CSF, the exception also showing full recovery upon discharge. Two of the four neonates' mothers had an unremarkable maternal history. One neonate's mother was febrile upon delivery and due to urinary tract infection, the mother of the last neonate expired 5 days after delivery due to preeclampsia, and an unknown febrile illness. The neonate whose mother expired was the one who had partially recovered from neurologic changes upon discharge. During the patients' hospital stay, there was no mention whether the mother was worked-up for the possibility of having acute dengue. It is yet to be established what the poor prognostic factors are for neonates presenting with dengue encephalitis, as there are limited studies regarding this.

Neurologic manifestations due to dengue have been well reported and has previously been thought to result from the multisystem derangement that occurs in severe dengue infection, with liver failure, shock and coagulopathy causing cerebral insultas opposed to encephalitis defined by a localized invasion of the CNS. Recent studies, however[10,11,13,14,21], describe a possible direct neurotropic effect of dengue virus. The incidence of dengue with neurologic complications is unclear, with calculations ranging from 0.5%[14] to 6.2%[26] of DHF cases. Kankirawatana et al. states that 18% of children with suspected encephalitis in a Thai hospital were found to have dengue infection. [27]

In the absence of a definitive histological examination of the brain, dengue encephalitis is exemplified by the identification of dengue specific antibodies or dengue antigen in the CSF. Detection of IgM in CSF is indicative of viral replication in CNS, but the titer is generally lower and short-lived when compared with serum, making it an unreliable marker. It is because of this that in previous studies, patients were considered as cases of dengue encephalitis when there is serologic evidence of dengue infection, coupled with focal neurologic manifestations or neuroimaging abnormalities. This consideration has also been employed in this study. In previous studies, mechanism of CNS infiltration has been observed via (1) virus-induced, cytokine-mediated breakdown of the blood-brain barrier, (2) via infiltration of virus-infected macrophages, or (3) by direct invasion of the virus itself. In accordance with these recent reports, we found 5 (35.7%) of 14 patients had dengue-specific IgM in the CSF, indicating a localized infection of the CNS. These patients consisted of 3 neonates, and 2 children. Of the 3 neonates, 2 recovered completely prior to discharge, with hospital stay of 8 and 41 days respectively. One neonate with IgM positive CSF exhibited fair activity prior to discharge. Two other children with IgM positive CSF both stayed at the hospital for 27 days, one was discharged with minimal verbal output and

occasional disorientation, and one exhibiting focal deficits and whom hypoxic-ischemic encephalopathy was also considered.

The clinical manifestations and investigatory findings in this study were consistent with those reported in the literature and reviews of dengue encephalitis. Fever was present in all cases. Following non-specific signs and symptoms, decreased sensorium and new onset seizures were the most common neurologic manifestations, the latter being the most common reason for consult and subsequent hospital admission. Elevation in liver enzymes, dengue-related nephropathy, glucose and electrolyte derangements, elevated prothrombin time, prolonged activated thromboplastin time, and signs of plasma leakage were seen in some of our patients. It has been well recognized that cerebral dysfunction may result from these findings and may account for some neurologic manifestations seen.CSF analysis of all patients exhibited normal results, a minority with slight hypoglycorrhachia and pleocytosis, all with absolute (100%) lymphocytosis, the findings of which were consistent with viral encephalitis in general. The most common EEG and neuroimaging findings were likewise consistent with dengue encephalitis.[10,11,13,14,21,27,35] Most patients manifested with generalized or focal background slowing via EEG, and neuroimaging findings ranged from being normal, to having evidence of cerebral edema, some with changes consistent with acute meningoencephalitis. Testing for correlation between established factors for poor prognosis, which were noted in some of the patients, such as extremes of age, under or over nutrition, presence of co-morbidities, signs of plasma leakage, hepatic involvement, and patient outcome could not be done due to the very limited sample size.

Among the flaviviridae, antigenic cross-reactivity appears to involve a group-reactive antigen shared by all members. In patients with previous Japanese encephalitis, these circulating low-titered antibodies may show cross-reactivity with dengue virus. Four patients who were initially treated as cases of dengue encephalitis were excluded due to the presence of Japanese encephalitis-specific IgM (3 cases) and Chikungunyavirus IgM (1 patient) in the CSF. Based on previous reports[10,11,13,14,21] and of the findings of this study, dengue infection encompasses an expanding clinical spectrum that rarely involves encephalitis due to a direct viral neurotropism.

Mortality due to dengue encephalitis varies from 5%[22] to 22%[14] in previous studies. The reported morbidity and mortality due to dengue encephalitis itself is low with most survivors recovering fully.[10,34,35] Documented sequelae from encephalitis included weakness, spasticity[35] and focal spasms[36]. Encephalitis accompanied by post-infectious neurological manifestations however may have a prolonged recovery. Our study limited our investigation to laboratory-confirmed dengue encephalitis in the absence of co-infection with other viruses in the CNS, and only a single mortality was observed. Neurologic manifestations were observed in 6 (42.9%) of patients upon discharge, ranging from mild to severe. The presence of long-term or permanent neurologic sequelae cannot be inferred since the only follow-up data available were the follow-up EEG of two patients, which showed improvement in the generalized background slowing in one, and a normal EEG in another patient taken 3 weeks from discharge. It would be interesting to know the long-term outcome of each patient using an established outcome scoring system on subsequent patient follow-up consults, so as to determine whether neurologic changes that have been present on discharge would lead to eventual recovery or deterioration. This exercise, however, is beyond the scope of this study.

According to the World Health Organization (WHO), the real burden of dengue encephalitis is under reported. Due to the potential risk for significant morbidity and mortality, it is recommended that appropriate awareness and preventive measures (environmental control for vector eradication and vaccination for children 9 years old and above) be done, and prompt case detection and subsequent management ensue. The small sample size, heterogeneity of clinical profile, and patient response are probably responsible for outcome variations.

CONCLUSION AND RECOMMENDATION

In conclusion, dengue encephalitis is emerging as an important, albeit rare entity. Patients' ages ranged from 3 days to 15 years, with a male to female ratio of 1:1.3. Most of the patients are from the National Capital Region. They are characterized by depression of consciousness and/or new onset seizures, metabolic or electrolyte imbalances, elevated liver enzymes and a risk for mortality. Those who survive have varied responses in that they may have temporary or permanent neurologic changes, or complete recovery. Pathophysiologic findings include generalized background slowing of electrical waveforms via EEG, cerebral edema as well as dengue-specific IgM in the CSF. Due to the potential morbidity and risk for death, it is important to entertain dengue encephalitis as a differential diagnosis in patients of all age groups, including neonates, who live in an endemic country, and presents with fever and neurologic changes.

At present, the burden of dengue encephalitis is underestimated. Support for programs that emphasize awareness and prevention, especially in endemic areas should be given importance. Patients with neurologic sequelae from dengue encephalitis should be assessed and reported accurately during regular follow-up consults. Immunization programs must be implemented and be made readily available. Establishment of referral systems and healthcare facilities that can adequately cater to dengue encephalitis patients is likewise important. It is recommended that large-scale prospective studies that will follow-up on the patient's long-term condition be performed for prognostication and more conclusive correlation between patient parameters and outcome.

BIBLIOGRAPHY

1. Liu JW, Khor BS, Lee CH, Lee IK, Chen RF, Yang KD. Dengue hemorrhagic fever in Taiwan. World Health Organization Dengue Bulletin. 2003; 27:3-24. 2.

2. Global Strategy for dengue prevention and control, 2012–2020. World Health Organization, Geneva, Switzerland, 2012. Accessed 10 Apr 2017 at http://apps.who.int/iris/bitstream/10665/75303/1/9789241504034_eng.pdf

3. Dengue and severe dengue (Fact sheet N°117). World Health Organization, Geneva, Switzerland, 2016 [cited 24 June 2016]. Available from: http://www.who.int/mediacentre/factsheets/fs117/en/

4. Republic of the Philippines Department of Health website. Accessed 15 Apr 2017.https://www.doh.gov.ph/sites/default/files/statistics/2016_Dengue_W47_1.pdf.

5. Tunkel AR, Glaser CA,Bloch KA, Sejvar JJ, Marra CM, Roos KL, Hartman BJ, Kaplan S, Scheld WM, Whitley RJ. The Management of Encephalitis: Clinical Practice Guidelines by the Infectious Diseases Society of America. CID Jun 2008:47.

6. World Health Organization (WHO) Regional Office for South-East Asia. Dengue: guidelines for diagnosis, treatment, prevention, and control. Special Programme for Research and Training in Tropical Diseases. 2009. Available from: 147. https://doi.org/WHO/HTM/NTD/DEN/2009.1

7. Global Strategy for dengue prevention and control, 2012–2020. World Health Organization, Geneva, Switzerland, 2012.Available from: http://apps.who.int/iris/bitstream/10665/75303/1/9789241504034_eng.pdf

8. Cherry JD, Harrison GJ, Kaplan SL, Steinbach, MD, Hotez PJ. Feigin and Cherry's Textbook of Pediatric Infectious Diseases, 7th ed. 2014. Elsevier Saunders, USA. Ch. 45.

9. M. States,W. Advi, T. Grade, et al.Dengue vaccine: WHO position paper – July 2016. 29 JULY 2016, 91th YEAR.World Health Organization weekly epidemiological record; p.350

10. Rao SM, Pradeep M, Dnyaneshwar M and Alai T. Dengue encephalitis – clinical spectrum and outcome. Intern Med Inside. 2013; 1:8. http://dx.doi.org/10.7243/2052-6954-1-8

11. Murthy JM. Neurological complications of dengue infection. Neurol India 2010; 58: 581-58.

12. Rao S, Kumar M, Ghosh S, Gadpayle AK. A rare case of dengue encephalitis. BMJ Case Rep 2013; doi: 10.1136/ber- 2012-008229. [4]

13. Deepak Madi et. al.Dengue Encephalitis – A Rare Manifestation of Dengue Fever.Asian Pacific Journal of Tropical Biomedicine 4, no. Suppl 1 (2014): S70-2 doi:10.12980/APJTB.4.2014C1006.

14. Cam BV, Fonsmark L, Hue NB, Phuong NT, Poulsen A, Heegaard ED. Prospective case-control study of encephalopathy in children with dengue hemorrhagic fever. *Am J Trop Med Hyg* 2001;

15. 65(6):848-51. https://www.ncbi.nlm.nih.gov/pubmed/11791985 (accessed 19 April 2017).

16. Peacock JL, Peacock PJ. Research design. (ed). Oxford handbook of Medical Statistics. United States: Oxford University Press; 2011. pp. 60-61.

17. Department of Health National Epidemiology Center Public Health Surveillance and Information Division. Field Health Services Information System (2013) Manila, Philippines. Annual Report.

18. Philippine Integrated Disease Surveillance and Response Report. (2014) Department of Health. Manila, Philippines. *Annual Report*.

19. Committee on Immunization. Pediatric Infectious Disease Society of the Philippines. (May 20, 2016). Statement on the use of dengue vaccine. Manila, Philippines.

20. Technical working group. Philippine Pediatrics Society. (October 18, 2010) 2010 Interim guidelines on fluid management of dengue fever and dengue hemorrhagic fever. Manila, Philippines.

21. Philippine Pediatric Society. Preventive Pediatric Health Care Handbook, eighth ed., 2016.

22. Samanta M, Kundu, CK, Guha G, Chattergee S. Unique Neurologic manifestations of Dengue Virus in Pediatric Population: A Case Series.*Journal of Tropical Pediatrics.*2012, Vol 58, No. 5.

23. Pancharoen C, Thisyakom U. Neurological manifestations in dengue patients.Southeast Asian J Trop Med Public Health 2001; 32:341-5.

24. Chin PS, Khoo PC, Hani, AW, Chem YK, Norizah I,Chua KB. Acute dengue in a neonate secondary to perinatal transmission.Med J Malaysia. 2008 Aug;63(3):265-6.

25. Chye JK, Lim CT, Ng KB, Lim JM, George R, Lam SK. Vertical transmission of dengue. Clin Infectious Diseases.1997

26. Porta M. A Dictionary of Epidemiology, 5th ed. NY. 2001. Oxford University Press; 5:p. 53.

27. Hendarto SK, Hadinegoro SR. Dengue encephalopathy. Acta Paediatr Jpn 1992;34:350-7.

28. Kankirawatana P, Chokephaibulkit K, Puthavathana P, Yoksan S, Somchai A, Pongthapisit V. Dengue infection presenting with central nervous system manifestation. J Child Neurol 2000;15;544-7.

29. Solomon T, Dung NM, Vaughn DW, Kneen R, Thao LT, Raengsakulrach B, *et al*. Neurological manifestations of dengue infection. Lancet 2000;355:1053-9.

30. Gulati S, Maheshwari A. Atypical manifestations of dengue. Trop Med Int Health 2007;12:1087-95.

31. Muzaffar J, Krishnan V, Gupta N, Kar P. Dengue encephalitis: why we need to identify this entity in a dengue-prone region. Sing Med J. Nov 2006. Vol.47: pp.975-7.

32. Administrative Order 2012-0006. Revised Dengue Clinical Case Management Guidelines 2011. Departmentof health of the Republic of the Philippines. 2012: 66-7.

33. Kliegman RM, Stanton BF, Geme GW III, Schor NF, De Maso DR, Denison MR, et al, editors. Nelson Textbook of Pediatrics. 20th ed. Philadelphia: Elsevier, Inc. 2016

34. Jackson ST, Hann Chu JJ, Chia P, Morgan OS, Ng LC. Dengue encephalitis. Intech. 2011:11-12. Available from:http://www.intechopen.com/books/flavivirus-encephalitis/dengue-encephalitis

35. Angibaud G,Luaute J, Laille M, Gaultier C. Brain involevement in dengue fever. J of clin neuroscience. Jan 2001, 8:63-5.

36. Pancharoen C, Thisyakorn U. Neurological manifestations in dengue patients. Southeast Asian J of Trop Med and Pub Health. Jun 2001, 32:341-5.

37. Soares C, Fairia L, Peralta LC, De Freitas MR, Pucchioni-Sohler M. Dengue infection: neulogical manifestations and cerebrospinal fluid analysis. J of Neurol sciences. Nov 2006, 249: 19-24.

DENGUE VACCINE ACCEPTANCE AND ASSOCIATED FACTORS AMONG PARENTS AND CAREGIVERS AT THE PHILIPPINE CHILDREN'S MEDICAL CENTER OUTPATIENT DEPARTMENT: A HOSPITAL-BASED CROSS-SECTIONAL SURVEY

CARLA MIA A. CARANDANG, MD
MICHAEL M. RESURRECCION, MD

ABSTRACT

BACKGROUND: The DOH has recently launched the first ever dengue vaccine that has successfully completed phase III clinical trials but an assessment of the general acceptance of the vaccine is widely lacking.

OBJECTIVES: This study determined the dengue vaccine acceptance and the factors associated with acceptance as well as the knowledge, attitudes and practices on dengue fever among parents and caregivers at the PCMC-OPD.

METHODS: A hospital-based cross-sectional survey was done at the PCMC-OPD using self-administered questionnaires regarding the KAP on dengue fever and vaccine acceptance. Multivariate analysis and Spearman's rank correlation were used to determine predictors of DV acceptance.

RESULTS: We found that DV acceptance among the participants was 81.3% (113 out of 139). Educational attainment, employment status, and monthly income are significantly associated with acceptance of dengue vaccine, and being female contributed to high acceptance. DV acceptance was strongly correlated with a lower income class. Educational attainment and employment status seem to affect DV acceptance but are not strong predictors.

CONCLUSIONS: The DV acceptance rate of the parents and caregivers of patients consulting at PCMC-OPD was high. The most important factors associated with acceptance are educational attainment, employment status and income class.

RECOMMENDATIONS: A similar study may be conducted with a larger population to study target populations in the Philippines. This kind of study can be utilized to formulate new strategies addressing the awareness and acceptance of the community for the new dengue vaccine.

KEYWORDS: Dengue, Dengue fever, Dengue vaccine acceptance, Dengue vaccination, Philippines

INTRODUCTION

Dengue is the fastest spreading mosquito-borne viral disease in the world. It is estimated that 50 million dengue infections occur each year around the world and approximately 2.5 billion people living in dengue-endemic countries in tropical and sub-tropical regions of the world are at risk of infection. The disease poses a serious public health problem that has dramatically increased in tropical regions of the world. It has also become a leading cause of child mortality in several Asian countries including the Philippines. The clinical presentations of dengue vary from asymptomatic infection to classical dengue fever (DF) and to more severe forms of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).

Dengue fever is a vector-borne illness that is endemic to the Philippines. According to the World Health Organization (WHO), it ranks as the most important mosquito-borne viral disease in the world. During the past five decades, the incidence of dengue has increased 30-fold. The Department of Health (DOH) reported 585, 324 cases of dengue in the Philippines from 2008-2012. It has a case fatality rate (CFR) of 0.55% or 3,195 deaths. The Philippines ranks fourth in the number of dengue cases among the 10 members of the Association of Southeast Asian Nations (ASEAN). The current surveillance system highlights hospitalized cases especially those with severe dengue manifestation. Ninety-six percent of all reported dengue cases from 2008-2012came from hospitalized settings, 57% of which was reported by the public sector. Fifty five percent were diagnosed with dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).

In the Philippines, suspected dengue cases from January 1- December 31, 2015 mostly came from Region III with 35,966 cases (CFR 0.13%) followed by Region IV-A with 33,709 cases (CFR 0.3%) and then NCR with 25,208 cases (CFR 0.44%). It was also noted that in the Philippines, males and females are equally affected by dengue fever. However, children 1-4 years of age are noted to have the highest case fatality rate.

The estimated economic and human burden of dengue in the Philippines is 18,074 disability adjusted life years (DALYs) with total annual aggregate cost of $345 million. This covers the cost of diagnosis, treatment and income loss for patients and caregivers. The disease burden can also affect the country's economy through loss of productivity caused by illness and pre-mature death, increased healthcare costs and possible reduction in tourism.

There is no specific dengue treatment and efforts for prevention have been utilized by the DOH. The preventive measures mainly focus on vector-control. The 4S Program is thoroughly promoted and well campaigned. However, despite the many efforts of the DOH and its partners, there is still a dramatic increase in the incidence of dengue from 2009 to 2010. The goal of the global strategy for dengue prevention and control in 2012-2020 is "to reduce the burden of dengue" and one of its technical element is future vaccine implementation. "The availability of a safe, efficacious and cost-effective vaccine would significantly alter the concept for dengue prevention"(WHO,2012). The DOH has recently come to the planning and implementation stage of the first ever dengue vaccine that has successfully completed phase III clinical trials.

An orientation and training workshop on the dengue vaccine implementation in public schools was held last February 2016 for healthcare teams and LGU representatives coming from NCR, Region IV-A and Region III. The vaccination was launched in public schools last April 2016. Dengue vaccination is considered a promising solution for dengue control, but its potential acceptability has not been assessed in our country. An assessment of the public's acceptance of the DV may influence the adaptation of vaccination strategies in certain regions and population groups. It is therefore important to assess the vaccine acceptance to generate recommendations for policy makers.

Despite having many studies on knowledge, attitudes, and practices (KAP) regarding dengue in the Philippines, there is a paucity of studies on the acceptance of dengue vaccine among the direct caretakers of children who bear the burden of the disease. Therefore, the goal of this research is to determine the acceptance of the dengue vaccine among the parents and caregivers to provide valuable information for the future of dengue vaccination.

The WHO reported that about 50% of the world's population is at risk for dengue. Asia is the most affected region with about 75% of the global burden of dengue. Reported hotspots in Asia are the following countries in decreasing order of cases:

1) Philippines 2) Thailand 3) Indonesia 4) Vietnam 5) Malaysia and 6) Singapore.

Dengue fever is a preventable disease. Updates on Dengue Prevention and Control Implementing vector control measures by providing the following commodities: chemical approaches and physical barrier (insecticide treated screen), 3) Strengthening advocacy strategies to heighten public awareness (i.e. Aksyon Barangay Kontra Dengue (ABAKADA), 4 o' clock habit and 4S Against Dengue), 4.) Updating of Dengue Manual of Operation (MOP) – aligning with the ongoing local and global development to include the five pillars: Program Management, Early Case Diagnosis and Management, Surveillance, Prevention and Control (Vaccine and Integrated Vector Management), Advocacy, Community, and Social Mobilization and 5) Introduction of Dengue Rapid Diagnostic Test (RDT) at the point of care (RHU level).

There is no specific treatment against dengue and vector control measures have limited utility, especially with the development of insecticide resistance among the mosquito population. Therefore, the availability of a dengue vaccine would be a major advance in the control of the disease.

With almost 50% of the world population at risk and an estimated annual burden that includes 230 million infections, 2 million severe cases and 25,000 deaths, dengue is considered a major public health concern that needs vaccination. After decades of research by different groups around the world, a vaccine for dengue has been developed.A consistent efficacy profile has been demonstrated for the candidate dengue vaccine in phase III efficacy studies over the 25-month efficacy phase. Findings showed that there is 65.6% reduction in symptomatic dengue due to any serotype. Moreover, there was noted 93.2% reduction in severe disease while there is 80.8% reduction in hospitalized dengue. This vaccine is a tetravalent dengue vaccine evaluated for protective efficacy in large scale trials in Latin America and Southeast Asia and is called the CYD tetravalent dengue vaccine (CYD TDV).

The studies on safety likewise had positive results from the 25-month active phase. There ported serious adverse events were consistent with medical disorders in the age group studied. The cumulative relative risk from each study shows that the candidate dengue vaccine reduced the risk of hospitalizations.

Primary physicians and nurses serve as the first-line healthcare providers of dengue case diagnosis, notification, and treatment. Knowledge, attitude, and practice (KAP) of primary HCPs regarding dengue disease also provide early recognition and improve the outcome of dengue control. In a study conducted in Taiwan with healthcare workers as participants, it was found that there is not enough acquaintance in notification timing and important clinical features of dengue disease. Future continued education is recommended on these parts to improve dengue control.

On the other hand, health and community development utilizes knowledge, attitudes and practices studies as a means of understanding the cognitive and behavioral aspects of preventive intervention. The KAP research design has been widely used to determine the present awareness of any kinds of diseases and practices and to test the effectiveness of any treatment or intervention program for any health-related issues. In a study by Kwon and Crizaldo, there has been a significant difference between knowledge and practice on dengue fever in a local community. Through various media sources, community members have moderate knowledge about dengue, yet it has not been put into practices.

Acceptance of the dengue vaccine is a critical factor for the success of any dengue vaccination program and a high level of acceptance is required. A study done by Harapan et. al in 2016 held in a small community in Indonesia showed a relatively high(75% acceptance) result. They also found that a good attitude toward dengue vaccine and vaccination practices were the most important independent predictors of higher dengue vaccine acceptance. A high monthly income and socioeconomic

status (SES) were also associated with better acceptance. A similar study was done by Hadisoemarto and Castro in 2013 however, they used the thought of a hypothetical vaccine to determine the acceptance and willingness to pay of participants in an urban area in Indonesia. They found that a future dengue vaccine can have a very high uptake even when delivered through the private market.

There is a paucity of studies regarding the KAP and acceptance of dengue vaccine in the Philippines. There has been no research tackling on the knowledge and acceptance of dengue vaccine in the local setting hence the purpose of this study.

METHODOLOGY

A cross-sectional study was carried out at the Philippine Children's Medical Center – Outpatient Department - Quezon City. The participants who enrolled in the study came from the PCMC charity and private clinics. Parents or guardians aged 18 and above were invited to answer the questionnaires after signing the informed consent.

The minimum sample size computed using Epi Info version 13 was at least 118 based on the percent (%) acceptance of caregivers of 77.8% adapted from the study of Harapan et al. (2016) in Indonesia.[12]The margin of error was set at 7.5% and confidence level at 95%. Purposive sampling method was employed and a total of 139 parents and caregivers participated in this study.

The participants were recruited in the PCMC-OPD and oriented by the principal investigator and a research assistant. The nature of the study was explained and all questions and inquiries from the participants were addressed. The subjects were asked to sign a voluntary informed consent after the investigator provided adequate opportunity for the participants to consider all options, answering their queries and by making sure that the participants fully comprehend the proceedings. The investigator explained the study thoroughly and gave the participants enough chance to raise questions before finally asking them to sign the informed consent form (see Appendix 1). The data from each subject were gathered using the General Information Sheet (see Appendix 2).The average time of completion of the questionnaire by the participants was 15 minutes. The data collection process took place for a total of seven days. The data collected were then processed and analyzed.

The questionnaires used for the KAP and acceptance were adapted from the study of Harapan et.al with due permission granted by the author himself. The questionnaires were composed of a total of 56 questions: a) 20 items for knowledge, b) 13 items for attitudes, c) 13 items for practices and d) 10 items for acceptance (see Appendix 2). These questions were in English and were translated in Filipino. The questionnaires were validated using both the foreign and local languages with the participation of the same population as the study. A reliability test of the questionnaires was done and a 0.7 cut-off point of Cronbach's alpha was applied indicating good internal consistency of the items.

Each valid response of knowledge regarding DF (signs, symptoms and transmission of dengue virus) was given a score of one while an incorrect response was scored as zero. The items within the attitude domain were answered by the participants using a five-point Likert scale. The possible responses ranged from "strongly disagree" to "strongly agree." A score of one to five could be received for each statement, and higher scores indicated a more positive attitude. The statements regarding the preventive practices against DF were also answerable by "yes" or "no". Each valid response of a measure to prevent mosquito-man contact and eliminate mosquito breeding sites were given a score of one. In the KAP domains presented, a higher score would indicate better knowledge, a more positive attitude and better preventive practices regarding DF. Inquiry for the dengue vaccine acceptance was done using a 10-item questionnaire with questions answerable by "yes" or "no". Each valid response for acceptance was given a score of one while a negative response was scored as zero. Acceptance was determined using the answer to

the statement, "I agree for my child/children to be given the dengue vaccine."

The data gathered were checked for completeness, accuracy and consistency. The data were encoded using MS Excel 2010. A corresponding control number was assigned to each response. Binary coding was employed for variables answerable by "yes" or "no". The categorical variables were coded using nominal numbers. The scores on the KAP and acceptance were recorded in percentage. Data analysis was performed in Stata SE version 13. Quantitative variables were summarized using mean and standard deviation and all qualitative variables were tabulated in frequency and percentage. Acceptance proportion was estimated with 95% confidence level and factors associated with acceptance were analyzed using logistic regression and Spearman's rank correlation.

RESULTS

A total of 139 subjects completed the questionnaires. Majority of the population was aged 30-41 years old (48.3%).Seventy-one percent were females and 56.1%was married. Most of the participants were either high school (46.8%) or college graduates (43.9%). Most subjects were unemployed (53.2%) and came from the lower income class (69.8%). Forty-one percent had one child. There were 17 participants (12.2%) with a child with prior history of dengue (Table 1).

Table 1. Frequency and percentage distribution of the sociodemographic characteristics of 139 participants.

Characteristics (N=139)	n (%)	Characteristics (N=139)	n (%)
Age		**Employment**	
18-29	37 (31.4)	Employed	65 (46.8)
30-41	57 (48.3)	Unemployed	74 (53.2)
42-53	17 (14.4)		
54 and above	7 (5.9)		
Gender		**Monthly income**	
Male	40 (28.8)	Upper class	22 (15.8)
Female	99 (71.2)	Middle class	20 (14.4)
		Lower class	97 (69.8)
Marital status		**No. of children**	
Single	55 (39.6)	1	57 (41.0)
Married	78 (56.1)	2	36 (25.9)
Separated	4 (2.9)	3	18 (12.9)
Widowed	2 (1.4)	≥4	28 (20.2)
Educational attainment		**History of dengue**	
Primary		Yes	17 (12.2)
Secondary	8 (5.7)	No	122 (87.8)
College	65 (46.8)		
Graduate level	61 (43.9)		
	5 (3.6)		

Table 2 outlines the mean and standard deviation of scores in the KAP. The mean percentage score for knowledge was further subcategorized as follows: transmission 85.8%, prevention 84.7% and signs and symptoms 85.4%. The attitude on dengue vaccine was determined among the participants. The mean score in the five-point Likert scale was 4.5. The mean percentage score of the participants in the practices against DF was 81.6

Table 2. Mean and standard deviation of the scores in the KAP.

Domains	mean ± SD
Knowledge	
Transmission (%)	85.8 ± 14.1
Prevention (%)	84.7 ± 23.3
Signs and symptoms (%)	85.4 ± 22.4
Attitude (5-point Likert scale)	4.5 ± 0.4
Practices (%)	81.6 ± 14.0

SD = standard deviation

Table 3 shows the frequency and percentage distribution of the associated factors with dengue vaccine acceptance. The overall acceptance of dengue vaccine was 81.3% or 113 out of 139 participants. Results showed that gender, educational attainment, employment status, and monthly income are significantly associated with the acceptance of dengue vaccine.

Table 3. Dengue vaccine acceptance and associated factors among the 139 participants.

Variables	Will accept (n=113), n (%)	Will not accept (n=26), n (%)	P-value
Age			
18-29	36 (31.9)	8 (30.8)	0.48
30-41	54 (47.8)	7 (26.9)	
42-53	17 (15.0)	9 (34.6)	
54 and above	6 (5.3)	2 (7.7)	
Gender			
Male	25 (22.1)	15 (57.7)	0.001
Female	88 (77.9)	11 (42.3)	
Marital status			
Single	40 (35.4)	15 (57.7)	0.126
Married	68 (60.1)	10 (38.5)	
Separated	3 (2.7)	1 (3.9)	
Widowed	2 (1.8)	0 (0)	
Educational attainment			
Primary	2 (1.8)	6 (23.1)	0.001
Secondary	52 (46.0)	13 (50.0)	
College	54 (47.8	7 (26.9)	
Graduate level	5 (4.4)	0 (0)	
Employment			
Employed	48 (42.5)	17 (65.4)	0.029
Unemployed	65 (57.5)	9 (34.6)	
Monthly income			
Upper class	4 (3.5)	18 (69.2)	<0.001
Middle class	17 (15.1)	3 (11.6)	
Lower class	92 (81.4)	5 (19.2)	
No. of children*	2.3 ± 1.4	1.8 ± 1.2	0.137
History of dengue			
Yes	14 (12.4)	3 (11.5)	0.604
No	99 (87.6)	23 (88.5)	

*mean ± SD

**Significant at P>0.05, Fisher's Exact Test

Using the independent t-test, the differences among qualitative variables were compared with acceptance. The mean number of children and the mean scores in the KAP domains do not significantly differ when compared with vaccine acceptance (Table 4).

Table 4. Mean and standard deviation of the factors and acceptance values.

Variables	Will accept (Mean ± SD)	Will not accept (Mean ± SD)	P-value
No. of children	2.3 ± 1.4	1.8 ± 1.2	0.14
Knowledge on transmission	85.1 ± 13.7	88.9 ± 15.9	0.21
Knowledge on prevention	84.6 ± 24.6	85.4 ± 16.5	0.88
Knowledge on signs and symptoms	85.6 ± 21.1	84.6 ± 27.7	0.84
Attitude	4.4 ± 0.45	4.5 ± 0.39	0.27
Practices	82.6 ± 13.1	77.2 ± 17.2	0.08

SD = standard deviation
*Significant at P<0.05, Independent t-test

A univariate analysis was done to determine the odds ratio of the associated factors to DV acceptance. Female parents and guardians are 4.8 times (95% CI 1.96-11.8, P<0.05)more likely to have their children vaccinated (Table 5). The data also showed that those who finished secondary and college education tend to have higher dengue vaccine acceptance. Those who are unemployed have higher dengue vaccine acceptance than those who are employed. Unemployed parents and guardians are 2.6 times more likely (96% CI 1.1-6.2, P<0.05) to have their children vaccinated than those who are employed. Similarly, those who belong to the lower income class have a significantly higher dengue acceptance.

Table 5. Univariate logistic regression analysis showing predictors of dengue vaccine acceptance.

Variables	n (%)	Percentage willing (%)	OR (95% CI)	P-value
AGE				
18-29	44 (31.7)	81.8	1.1 (0.42-2.6)	0.91
30-41	62 (44.6)	87.1	2.06 (0.83-5.1)	0.12
42-53	25 (18.0)	68.0	0.40 (0.15-1.1)	0.06
54 and above	8 (5.7)	75.0	0.67 (0.13-3.5)	0.64
GENDER				
Male	40 (71.2)	62.5	0.21 (0.09-0.51)	0.0006
Female	99 (28.8)	88.9	4.8 (1.96-11.8)	0.0006
EDUCATION				
Primary	8 (5.7)	25.0	0.08 (0.02-0.46)	0.0044
Secondary	65 (46.8)	80.0	0.85 (0.36-2.0)	0.71
College graduate	61 (43.9)	88.5	1.92 (0.71-5.2)	0.20
Graduate level	5 (3.6)	100	1	
EMPLOYMENT STATUS				
Employed	65 (46.8)	73.8	0.39 (0.16-0.95)	0.04
Unemployed	74 (53.2)	87.8	2.6 (1.1-6.2)	0.04
MARITAL STATUS				
Single	55 (39.6)	72.7	0.39 (0.16-0.96)	0.04
Married	78 (56.1)	87.2	2.4 (1.0-5.8)	0.04
Separated	4 (2.9)	75.0	0.44 (0.04-4.7)	0.50
Widowed	2 (1.4)	100	1	

Table 5. (Continuation)

Variables	n (%)	Percentage willing (%)	OR (95% CI)	P-value
MONTHLY INCOME				
Upper class	22 (15.8)	18.2	0.01(0.003-0.05)	<0.0001
Middle class	20 (14.4)	85	0.31 (0.07-1.4)	0.13
Lower class	97 (69.8)	94.8	18.4 (6.2-54.4)	<0.0001
HISTORY OF DENGUE IN A CHILD				
Yes	17 (12.2)	82.4	1.08 (0.29-4.1)	0.91
No	122 (87.8)	81.1	0.92 (0.24-3.5)	0.91

OR = odds ratio, CI = confidence interval
*Significant at P<0.05

Furthermore, a multivariate analysis was done to address the confounding effect of educational attainment, employment status and income class (Table 6). Results showed that the independent factors associated with dengue vaccine were educational attainment and income class.

Table 6. Multivariate logistic regression analysis showing predictors of dengue vaccine acceptance.

VARIABLES	aOR (95% CI)	P-value
GENDER		
Male	0.77 (0.13-4.4)	0.35
Female	1	
EDUCATIONAL ATTAINMENT		
Primary	0.22 (0.01-4.1)	
Secondary	3.4 (0.33-33.8)	<0.0001
College graduate	2.3 (0.41-12.6)	
Graduate level	1	
EMPLOYMENT STATUS		
Employed	0.50 (0.08-3.2)	0.77
Unemployed	1	
MONTHLY INCOME		
Upper class	1	<0.0001
Middle class	0.35 (0.05-2.4)	
Lower class	2.8 (0.06-5.1)	

aOR = adjusted odds ratio, CI = confidence interval
*Significant at P<0.05

The correlation of DV acceptance with marital status is weak while the correlation between higher education and dengue acceptance was positively significant (r = 0.34, 95% CI 0.18-0.48, P<0.05). Similarly, a lower income class showed a moderate correlation with dengue acceptance (r = 0.69, 95% CI 0.59-0.77, P<0.05).The mean score on knowledge showed a weak negative correlation with dengue vaccine acceptance while the other two domains showed no significant correlation.

DISCUSSION

The tetravalent dengue vaccine (CYD-TDV) has been launched in the Philippines recently and has been implemented via school-based immunization programs in Regions III, IV-A and NCR. It is thus important to understand its acceptance and associated factors among the parents and guardians of the most susceptible population, the children. This study was conducted to elucidate the dengue vaccine acceptance of the parents and guardians consulting at PCMC and to determine the potential determinants of vaccine acceptance.

This study showed that the parents and caregivers at PCMC-OPD have a very good acceptance of dengue vaccine which was 81.3%. The most important associated factors for acceptance of the DV were educational attainment, employment status and income class. The rate of DV acceptance in this study is comparable to the three different studies conducted in Aceh (70% and 77.3%)[12,] and Bandung, Indonesia (94.2%).[13]

The scores in the KAP domains did not significantly affect the DV acceptance. The knowledge domain however had a weak negative correlation while the other domains showed no significant correlation. This result agrees with that of Harapan et. al which showed that knowledge of a disease did not have a discernible effect on increased vaccine acceptance. These findings may suggest that a better understanding of a disease without enhancing the attitude or prevention practice does not increase vaccine acceptance.[12]

We found no association between DV acceptance and attitude and practices. This is contrary to previous studies (Harapan et al., 2016 & Dhimal et al., 2014) which showed that good attitude towards dengue fever and good participation in its prevention correlate with better DV acceptance.[16,17]In these studies, good attitudes toward DF increased DV acceptance by approximately four times. The disparity of results may be since most responses garnered from these domains in the study have high scores thereby masking the effect of the responses of the minority. This may be improved by further increasing the sample size.

Females have significantly higher acceptance for dengue vaccine (OR: 4.8 95% CI 1.96-11.8, P<0.05). The result of this study is like the study done in Aceh, Indonesia in which being female increased the odds of having good attitude towards vaccination more than two times than males.[16] This result may be influenced by the socially constructed roles and responsibilities of males and females. Females play a major role in domestic works which includes caring of ill members of the family including children which gives way to a positive attitude towards vaccination. However, the results of our study must be interpreted cautiously due to the fact that 71.2% of the population were females and they may have provided socially desirable responses.

Furthermore, we found that educational attainment, employment status and income class were significantly associated with DV acceptance. Income class which is reflective of the socioeconomic status, is likewise associated with educational attainment and employment.[13] The confounding effect may have masked the true association between income class and DV acceptance. This was addressed by doing the multiple logistic regression analysis which revealed that only educational attainment and employment status were the independent factors.

A high level of education was found to significantly predict higher DV acceptance. This result supports some studies showing educational attainment to be associated with better attitude toward dengue vaccine[13] and that a low level of education was a barrier for vaccine acceptance.[12]However, the studies of Hadisoemarto and Harapan both showed that education was not a strong factor in DV acceptance. They found that individuals who completed secondary education were less likely to support DV compared to those with lower education.[13,16] This association was not observed among the participants with a college degree or higher. Education therefore is considered an intermediate factor that is affected and affects other factors as there are other studies on

vaccination acceptance that show conflicting results of higher education as a predictor.

The members of the lower income class have a higher DV acceptance than those from the richer population. This is contrary to the previously established result from other studies showing that a high income level and socioeconomic status (SES) were associated with better DV acceptance.[15] As SES was found to be a weak explanatory factor, they also found that the DV acceptance between the richest and the poorest groups did not significantly differ.[18]In studies done in the USA, Nigeria, Burkina Faso and India, SES and income class have strong association with better vaccine acceptance while other studies in the same regions also found it to be associated with low vaccine acceptance.[15,] A low SES has been linked to issues of trust in healthcare providers and is also related to low education level and low access to vaccine. The reasons why SES and income class are associated with vaccine acceptance are not always explained. It was suggested that wealthier people tend to be more likely to accept DV because they are more likely to spend money for the vaccine while the opposite is true for those who belong to the lower income class.[18]Given that majority of our participants came from the lower income class and more people accepted the vaccine, this might have significantly tipped the scales towards significance.

In this study, we found that the parents and guardians who are unemployed have higher dengue vaccine acceptance than those who are employed. Employment status is influenced most of the time by the educational attainment of an individual.[18] As educational attainment and employment were confounding factors for SES, we assume that unemployment is associated to a lower income class thus both factors predicted a high DV acceptance in our study. However, based on the correlation analysis, employment status has a weak correlation and is inconsistently associated with acceptance. This makes both income class and employment status inconsistent predictors for DV acceptance. We found no significant association between a personal history of DF in a child and DV acceptance. This finding is the same with the study in Aceh, Indonesia where they found that participants who had direct personal experience with DF and those who had a family member with history of DF had no association with DV acceptance.[12]In contrast to a similar study done by Hadisoemarto and Castro in 2016, they found that those with personal experience with dengue are more likely to accept the vaccine. This was explained by the fact that these parents were able to weigh the possible benefits of vaccinating against dengue because of the perceived risk of having a child getting the disease.[13]Our results can be explained by the premise that the DV was safe and protective against DF and that no information related to the dose, administration procedure or the price of the DV were provided to the participants. We also did not determine the severity of the previous dengue infection for those who had an experience with DF. Therefore, the actual acceptance may have not been reflected in our results.

The findings of this study can contribute data for formulating a suitable program for an accelerated introduction of dengue vaccines. Utmost efforts by the government should be done to further disseminate information regarding the vaccine especially for those who have a lower level of education and are poor. In order to improve vaccine acceptance both the communicative skills of healthcare workers and proper information about dengue vaccination are crucial. A deeper understanding on the vaccine may enhance trust of the community member among the healthcare workers which brings about higher DV acceptance.

CONCLUSION AND RECOMMENDATIONS

The DV acceptance rate of the parents and caregivers of patients consulting at PCMC-OPD was 81.3%. The most important factors associated with acceptance are educational attainment and income class. Female gender is associated with high DV acceptance. Knowledge is associated with better support for dengue vaccination but is not a strong predictor. Attitude and practices on DF have no association

with DV acceptance. A previous experience with DF is not a significant factor that boosts DV acceptance.

In future, this kind of study may be conducted on a larger population involving different regions or municipalities in the Philippines especially in target places with high dengue cases. This can help determine the true picture of awareness and acceptance of the community for the recently launched dengue vaccine. New studies on KAP and vaccine acceptance can help improve the attitudes and practices toward DF and vaccination thereby improving acceptance. We propose that new strategies to improve the knowledge, attitude and practices be done targeting focus groups of parents and caregivers who belong to a higher SES, higher level of education and are employed. The results of this study can aid the policy makers to formulate new strategies addressing the awareness and acceptance of the community for the new dengue vaccine.

BIBLIOGRAPHY

1. Global strategy for dengue prevention and control 2012-2020. WHO 2012.

2. Economic cost and burden of dengue in the Philippines. *Am J Trop Med Hyg.* 92(2), 2015, pp.360-366.

3. World Health Organization Western Pacific Region, 2013.Emerging Disease Surveillance and Response: Dengue Situation Updates. WPRO 2014. Available at:http://www.wpro.who.int/emerging_diseases/DengueSituationUpdates/en/. Accessed February 8, 2016.

4. Edillo F, Madarieta S, 2012. Trends of dengue infections (1997–2008) in Cebu Province, Philippines.Dengue Bull 36:37–49.

5. National Epidemiology Center of the Department of Health, 2008.Manual of Procedures for the Philippine Integrated Disease Surveillance and Response. Manila, Philippines: Department of Health.

6. Amarasinghe A, Wichmann O, Margolis HS, Mahoney RT. Forecasting dengue vaccine demand in disease endemic and non-endemic countries. Hum Vaccin. 2010;6:745-53.

7. Borja M, 2007.Final Report of Burden of Dengue in the Philippines. Manila, Philippines: Department of Health.

8. Kwon, DH and Crizaldo, RL. A knowledge, attitudes and practices (KAP) study on dengue fever among the Rowenas Community in the Philippines. *Mediator*, 10:1 (2014), pp 1-21.

9. DOH Dengue Trends in the Philippines 2015.

10. Halstead SB, Heinz FX. Dengue virus: molecular basis of cell entry and pathogenesis, 25-27 June 2003, Vienna, Austria.Vaccine, 2005, 23(7):849--856.

11. Tzong-Shiann, H, MC Huang, SM Wang, et al. Knowledge, attitude, and practice of dengue disease among healthcare professionals in southern Taiwan. *Journ of the Formosan Med Assoc*, 112:18-23 (2013).

12. Harapan, H, S Anwar, AM Setiawan, et al. Dengue vaccine acceptance and associated factors in Indonesia:A community-based cross-sectional survey in Aceh. *Vaccine*, 34:3670-3675 (2016).

13. Hadisoemarto, PF and MC Castro. Public Acceptance and Willingness-to-Pay for a Future Dengue Vaccine: A Community-Based Survey in Bandung, Indonesia. *PLOS Neglected Tropical Diseases*, 7(9):24-27 (2016).

14. Harapan, H, S Anwar, AM Setiawan, et al.Modifiable determinants of attitude towards dengue vaccination among healthy inhabitants of Aceh, Indonesia: Findings from a community-based survey. *Asian Pacific Journ Trop Med,* 360:1-8 (2016).

15. Dhimal M, Aryal KK, Dhimal ML, Gautam I, et al. Knowledge, attitude and practice regarding dengue fever among the healthy population of highland and lowland communities in central Nepal. *PLoS One* 2014; 9(7): e102028.

16. Larson HJ, Jarrett C, Eckersberger E, Smith DM, Paterson P. Understanding vaccine hesitancy around vaccines and vaccination from a global perspective: a systematic review of published literature, 2007–2012. *Vaccine*2014;32:2150–9.

17. Khan AA, Varan AK, Esteves-Jaramillo A, Siddiqui M, Sultana S, Ali AS, et al.Influenza vaccine acceptance among pregnant women in urban slum areas,Karachi, Pakistan. *Vaccine* 2015;33:5103–9.

THE ELECTROLYTE PROFILE AMONG PEDIATRIC PATIENTS WITH DENGUE FEVER ADMITTED IN PHILIPPINE CHILDREN'S MEDICAL CENTER

MARY ANGELI A. CONTI, MD
MARY ANTONETTE CUADY-MADRID, MD

ABSTRACT

OBJECTIVE: To determine the electrolyte profile (Sodium, Potassium and Calcium) of non-shock dengue patients admitted at Philippine Children's Medical Center in 2012.

DESIGN: Retrospective cross-sectional study. Chart review was done, each of which underwent 3-stage screening process, aided by a data-collection form, to confirm the diagnosis based on the WHO Criteria.

MAIN OUTCOME MEASURES: Electrolyte profile and prevalence of electrolyte derangements

RESULTS: The mean sodium, potassium and calcium levels among non-shock pediatric dengue levels are 136.87 ±0.03 meq/L, 4.01 meq/L and 2.09 mmol/L respectively. The most common electrolyte disturbance ishypocalcemia occurring in ~69% of patients. The prevalence of hyponatremia and hypokalemia were ~27% and ~8% respectively. All electrolyte abnormalities were frequently observed in the >6year to 12 years age group.

CONCLUSIONS: Hyponatremia, hypokalemia and hypocalcemia were observed among the non-shock dengue patients in this study, however only hypokalemia and hypocalcemia were shown to be clinically significant.

RECOMMENDATIONS: Having established the electrolyte trend among non-shock dengue patients, controlled cohort studies that show association to disease outcome is recommended. Since this study showed significant results on the prevalence of electrolyte disturbances across all age groups, metabolic derangements may contribute to significant morbidities. Thus these should be addressed in symptomatic patients.

KEYWORDS: Electrolyte profile, Non-shock Dengue, Hyponatremia in Dengue, Hypokalemia in Dengue, Hypocalcemia in dengue

INTRODUCTION

Dengue is the most rapidly spreading mosquito-borne viral disease in the world. In the last 50 years, incidence has increased 30-fold with increasing geographic expansion to new countries and, in the present decade, from urban to rural settings. Dengue infection is a systemic and dynamic disease with wide clinical spectrum that includes both severe and non-severe clinical manifestations. This complex disease has a relatively simple management so long as correct and timely interventions are instituted. The key is early recognition and understanding of the clinical problems during the different phases of the disease, leading to a rational approach to case management and a good clinical outcome.

Studies on the biochemical profiles have been done but with limited data on electrolytes specifically that of calcium. Issues on the electrolyte profile during the early course of the

disease has not been established, hence no targeted management for electrolyte imbalances is reflected in the WHO protocol.

According to Malavige et al., reported electrolyte imbalances were as high as 80% among dengue patients. This being a contributing factor to one of worst outcome in dengue, encephalopathy, establishing a profile by age group is needed.

In a study by Faridi et. al., there was no significant difference in serum electrolytes including serum calcium, according to the age or sex of the children. As it was a descriptive study involving only 34 patients, of 0-12 age group, an analytic study with a larger sample size will hope to establish significant results on actual profile of electrolytes. This study has been designed to establish a profile on the electrolytes (sodium, potassium, calcium) among children with dengue patients. Significant results may aid in assessing the need for immediate intervention among non-shock patients with a possibility of a better disease outcome.

The most common documented electrolyte abnormality in Dengue is hyponatremia. This was evident in the study done by Lumpaopong where 61% and 72% of patients with Dengue fever and Dengue hemorrhagic fever, respectively was observed. Another study involving infants with dengue, 54.7% of the patients had hyponatremia. This abnormality of hyponatremia may be due to a decrease in salt intake, an increase in tubular reabsorption and an increase antidiuretic hormone secretion secondary to stress, fever or dehydration.

Hyponatremia has been correlated with disease severity as well, according to Isturiz et. al. This was also supported by a study from India, where patients with significant hyponatremia had poorer outcome. This then triggers a query if early detection and correction can significantly change the course of the disease in becoming more severe.

Mild hypokalemia was documented among Dengue patients, ~15% was reported. A lower percentage was seen in a study in 2004, where only 4.7% had hypokalemia. The difference in the result may be due to the age group involved in the two studies, the latter only focusing on infants, while the former included all patients from 0-18 years of age. Primarily, hypokalemia among Dengue patients may be due to poor intake and an increase in renal excretion due to activation of renin, angiotensin and aldosterone system secondary to volume depletion.

There has been a scarcity of literature reporting on hypocalcemia as a complicating factor in dengue, it is an infrequently recognized complication. Through a study by Nguyen, only 4.7% of the patients had hypocalcemia, although the profiles of the 2patients were not expounded on. Most researches on dengue profiles which included serum calcium levels are limited to those studies involving cases of shock and those in the ICU set-up. In a published report, a significant number of patients had hypocalcemia, and some of them weres ymptomatic. The pathophysiology that may contribute to hypocalcemia is not well understood. Analyses of Calcium binding to albumin in the serum of ICU patients and normal subjects suggested that there is a circulating factor in critically ill patients that increases the binding of Ca to albumin, this becoming the basis for the trend in shock patients.

OBJECTIVE OF THE STUDY

General Objective

To determine the electrolyte profile of non-shock dengue patients admitted at Philippine Children's Medical Center in 2012.

Specific Objectives

1. To determine the serum sodium, potassium and calcium levels among children with dengue by age group.

2. To determine the prevalence of hyponatremia, hypokalemia and hypocalcemia among children with dengue.

METHODOLOGY

A cross sectional design was employed for this study. A retrospective review of the medical records of patients with non- shock Dengue who were admitted at PCMC was carried out. The target population for this study included all patients, 0-18 years of age, admitted at Philippine Children's Medical Center from January 2012- December 2012 with a diagnosis of Dengue Hemorrhagic Fever I and Dengue Hemorrhagic Fever II.

Non-shock dengue patients whose serum electrolyte levels were taken prior to infusion of intravenous fluids were included in the study. They must also satisfy the WHO Criteria for Dengue Fever.

The following subjects were excluded from this study:

- DHF III and DHF IV cases
- Patients with previous intravenous hydration from another institution prior to admission at PCMC for this disease
- Patients whose serum electrolyte levels were determined after 24 hours of IV hydration
- Patients with co-morbidities

To estimate for the sample size, this formula was used $\frac{Z_{1-\alpha/2}^2 p(1-p)}{d^2}$. This study allowed a maximum error rate of 20%, thus also having an 80% power.95% confidence interval was used (z=1.96).The expected proportion in the population (p) was based on previous studies as seen in the table below. The sample size for every variable were adjusted based on the population size in Philippine Children's Medical Center. Since the variable on hyponatremia had the highest number of computed size, the minimum sample requirement was based on this figure.

Chart review was done to complete the data needed for analysis. We pooled the charts of all admitted cases of non- shock dengue patients from the Records Department of the institution. There were 850 charts screened for eligibility to be included in the study.

An algorithm was followed to establish a sample's eligibility for study inclusion. The algorithm and data collection form was designed based on the 2009 WHO guideline for the diagnosis of Dengue Fever.

There were three screening processes done in this study. First was to scan for the availability of the electrolyte results. The second and third screening processes were conducted using a data collection tool.

The investigator utilized a data collection form for screening and data collection, it is , divided into 3 blocks. Block A included information on demographics and data on the timing of intravenous fluid infusion. Block B was used for the second screening process, it was centered on confirming the patient's diagnosis of dengue. This block had three subsections as follows 1) diagnosis, 2) checklist based on the WHO criteria for dengue, and 3) laboratory work-up. Patients who were not able to meet the WHO criteria in establishing Dengue fever were excluded in the study. Block C contained electrolyte results and the timing of its extraction. This confirmed if electrolyte determination was done prior to IVF infusion, otherwise the sample was excluded.

The information gathered was checked for completeness. These were encoded in a mother table using the Excel program. This was facilitated using a coding manual that was initially prepared. Completeness and consistency of inputs were checked. Summaries were presented in the form of tables.

The data was encoded in a mother table using the Excel program. The mean was computed using the same program at a 95% confidence interval. The frequencies and prevalence among age group were computed.

Chi square test was used to compute for its statistical significance.

RESULTS

A total of 213 charts were included in the study. There was an almost equal distribution of patients as to sex, with 50.7% males and 49.3% females. Most of the participants belonged to the 6 to <12 years age group (41.3%). The age and sex distribution of included patients is summarized in table 1.

Table 1. Age-Sex Distribution Table of Non-Shock Dengue Patients Admitted at Philippine Children's Medical Center in 2012

Age group	Male		Female		Total	
	Frequency (%)	Percent	Frequency	Percent	Frequency	Percent
<12 months	7	3.4%	7	3.4%	14	6.6%
1 year – < 6 years	35	16.8%	29	13.9%	64	30.0%
6 – <12 years old	39	18.8%	49	23.6%	88	41.3%
12 – 18 years old	27	13.0%	20	9.6%	47	22.1%
Total	108	50.7%	105	49.3%	213	100%

As this study included all non-shock dengue cases, this may be further classified. Fifty eight percent [58.2% (n/N=121/213)] of the participants were diagnosed as Dengue Hemorrhagic Fever 1 and 41.8% were diagnosed as Dengue Hemorrhagic Fever 2.

Cumulatively, serum sodium levels among all age group averaged 136.87 ±0.03meq/L. Table 2 shows a summary of sodium levels categorized by age group.

Table 2. Mean Levels of Sodium by Age Group among Non-Shock Dengue Patients Admitted at Philippine Children's Medical Center in 2012

Age group	Normal Values	Serum Sodium (meq/L)
	mmol/L	Mean (95% CI)
<12mo	134-144	136.07 ±0.77
12mo- 2yr	134-143	138.00 ±0.59
>2yr	135-145	136.30 ±0.14
6-11 yr.	135-145	137.10 ±0.07
12-18 yr.	135-145	137.10 ±0.14

The prevalence of sodium derangements across all ages is 30.0% (n/N= 64/213), and 27.2% are hyponatremic (n/N=58/213). The frequency of these derangements by age group is seen in Table 3. A chi-square test showed a p-value of 0.323, hence there is no sufficient evidence to conclude that the prevalence of sodium derangements differs among age group.

Table 3. Prevalence of Hyponatremia and Hypernatremia Among Non-Shock Dengue Patients Admitted at Philippine Children's Medical Center in 2012

Age group	Serum Sodium Status n= frequency (%) Percent			Total
	Hyponatremia	Normal	Hypernatremia	
<12mo	5 (2.35)	8 (3.76)	1 (0.47)	14 (6.57)
12mo- 2yr	1 (0.47)	9 (4.23)	1 (0.47)	11 (5.16)
> 2yr – 6yr	19 (8.92)	33 (15.49)	1 (0.47)	53 (24.88)
> 6-12 yr	22 (10.33)	61 (28.64)	5 (2.35)	88 (41.34)
> 12-18 yr	11 (5.16)	36 (16.90)	0 (0.00)	47 (22.07)
Total	58 (27.23)	147 (69.01)	8 (3.8)	213 (100)

Among five age groups, there are different normal values set for its serum levels. In a population of 213, the mean potassium level was 4.01 meq/L. Table 4 below summarizes the actual potassium levels by age group

Table 4. Mean levels of Potassium by Age Group among Non-Shock Dengue Patients Admitted at Philippine Children's Medical Center in 2012

Age group	Normal Values	Serum Potassium (meq/L)
	mmol/L	Mean(95% CI)
0 - 6 months	3.5-5.6	4.70± 0.32
6 months- 1 year	3.5-6.1	4.68± 0.07
1 year – 6 years	3.3-4.6	4.14± 0.01
> 6-12 years	3.3-4.6	3.86± 0.01
>12-18 years	3.3-4.6	3.90± 0.02

In terms of serum potassium, the prevalence for abnormal levels is 16% (n/N= 34/213) and each half were hypokalemic and hyperkalemic (8%), respectively. The highest frequency of hyperkalemia was seen in the >1 yr to 6 years age group, while hypokalemia had increased frequency in the >6-12 year age group. There was significant statistical difference in potassium levels among the different age group. The frequency of the serum potassium status by age group is seen in Table 5.The p-value computed from the chi-square test leads us to say that there is statistically significant difference among age groups.

Table 5. Prevalence of Hypokalemia and Hyponatremia By Age Group among Non-Shock Dengue Patients Admitted at Philippine Children's Medical Center in 2012

Age group	Serum Potassium Status n= frequency (%) Percent			Total
	Hypokalemia	Normal	Hyperkalemia	
0 - 1 year	0 (0.00)	15 (7.04)	0 (0.00)	15 (7.04)
>1 year – 6 years	3 (1.41)	52 (24.41)	11 (5.16)	66 (30.99)
> 6-12 years	8 (3.76)	73 (34.27)	4 (1.88)	85 (39.91)
>12-18 years	6 (2.82)	39 (18.31)	2 (0.94)	47 (22.07)
Total	17 (7.98)	179 (84.04)	17 (7.98)	213 (100)

Serum calcium levels among all age group averaged 2.09 mmol/L. Table 6 shows a summary of calcium levels categorized by age group.

Table 6. Mean levels of Calcium by Age Group among Non-Shock Dengue Patients Admitted at Philippine Children's Medical Center in 2012

Age group	Normal values	Serum Calcium (mmol/L)
	mmol/L	Mean (95% CI)
0- 2 years	2.25-2.75	2.07 ± 0.17
> 2- 6 years	2.2-2.7	2.09 ± 0.16
>6-12 years	2.2-2.7	2.07 ± 0.14
>12-18	2.1-2.55	2.1 ± 0.14

More than 60% of the included subjects who had hypocalcemia belonged to the >6-12-year age group. Differences in calcium levels among the different age group was statistically significant.

Table 7. Prevalence of Hypocalcemia by Age Group among Non-Shock Dengue Patients Admitted at Philippine Children's Medical Center in 2012

Age group	Serum Calcium Status n= frequency (%) Percent		Total
	Hypocalcemia	Normal	
0- 2 years	13 (6.10)	12 (5.63)	25 (11.74)
> 2- 6 years	42 (19.72)	14 (6.57)	56 (26.29)
>6-12 years	69 (32.39)	16 (7.51)	85 (39.99)
>12-18	23 (10.80)	24 (11.27)	47 (22.07)
Total	147 (69.01)	66 (30.99)	213 (100.00)

X^2 (3, N=213) = 13.8, p<0.001

DISCUSSION

Dengue is a progressing disease burden in our country today and there has been a wide array of research that has been conducted to identify its clinical features. However, information on the electrolytes of dengue patients is currently limited. This study established that electrolyte derangement is a cause for concern for dengue. It is present in almost 80% of cases according to a study by Malavige. In this study, it is as high as 69% particularly for hypocalcemia. Patients may even have multiple electrolyte derangements at the onset of the disease which may contribute to the course and outcome of the disease.

Previous studies have established that the most common electrolyte derangement in dengue is hyponatremia, as supported by a study by Lumpaopong where 61% and 72% of patients with Dengue fever and Dengue hemorrhagic fever, respectively was observed. In our study, the prevalence of hyponatremia is only 27.23%. Sodium levels among dengue patients were documented to be 126.47±9.26, clinically significant compared to a control group as seen in the study by Syed, with no particular age group specified. This is low compared to the mean levels of sodium in the present study as seen in table 3. The low frequency of hyponatremia could be explained in part by the widely accepted use of ORS among suspected dengue cases currently. Once signs and symptoms for hospitalization are observed, further work-up is done and that the electrolytes level may not be a reflection of the true sodium levels for dengue cases. The etiology for hyponatremia as described by other studies is mainly due to decrease in salt intake, increase in tubular re-absorption and increase in anti-diuretic hormone secretion secondary to stress, or dehydration. This may be affected by the initial oral hydration recommended in our institution.

This study showed a lower prevalence of hypokalemia (~8%, p-value 0.035) compared to another study where hypokalemia was 17%, seen in 77 patients. The mean levels of serum potassium from previous studies among children with dengue was 3.89 ± 0.42, which was generally lower compared to the mean levels observed among different age group in this study as seen in Table 5. The latter study was not statistically significant perhaps because of a smaller sample population.

Hypocalcemia as a complicating factor in dengue, is an infrequently recognized complication. In a study by Nguyen, only 4.7%

of the patients had hypocalcemia,while this study showed a higher prevalence (69%, p-value <0.0001). Although there is a significantly high prevalence in hypocalcemia, its pathophysiology is not well understood as there is paucity of clinical evidence on the role of calcium disequilibrium in dengue. Analyses of calcium binding to albumin suggests that there is a circulating factor in critically ill patients that promotes the increased Calcium binding to albumin. The mean ionized serum calcium level of dengue patients from a study by Constantine is 1.05 mmol/L (range 0.77–1.24).The present study showed a slightly higher range as seen in Table 7. These values are not comparable due to the difference of age group that each study covered and that the previous study was able to document ionized serum calcium levels, in contrast to the present study's documentation of total calcium levels. As ionized calcium is a more useful index than total calcium and it provides a better indication of calcium status, this point becomes one of the present's studies limitations.

In the process of achieving this study's objectives, the researcher experienced difficulties particularly in data collection. Ideally, random sampling was to be done, but during the course of data collection the primary inclusion criteria is the availability of the information on serum electrolytes. The sampling frame of 850 was used for which all were screened for the availability of the information being gathered. This being the case, the generalizability of the result may be compromised as well.

CONCLUSIONS AND RECOMMENDATIONS

The mean sodium, potassium and calcium levels among non-shock pediatric dengue levels are 136.87 ±0.03 meq/L, 4.01 meq/L and 2.09 mmol/L respectively. Hyponatremia, hypokalemia and hypocalcemia were observed among the non-shock dengue patients in this study, however only hypokalemia and hypocalcemia were shown to be clinically significant. Of the three, hypocalcemia was the most common electrolyte disturbance occurring in ~69% of patients. The prevalence of hyponatremia and hypokalemia were ~27% and ~8%, respectively. All electrolyte abnormalities were frequently observed in the >6year to 12 years age group.

It is essential to establish a good electrolyte profile for dengue patients to aid in assessing the need for immediate intervention among non-shock patients since clinical course and outcome can be affected. With that in mind, studies on association of the prevalence of electrolyte derangements and disease outcome may help achieve this goal by using a controlled cohort study.

As there are different range of normal values per age group, classifying the severity of electrolyte derangements in association to disease outcome may be advocated for succeeding studies.

One of the major limitations of this study is the documentation of total serum calcium which is a less reliable measure of the true calcium levels as compared to ionized calcium. It is then recommended that electrolyte profiling of ionized calcium levels be pursued.

In this study, the reported prevalence of hypokalemia and hypocalcemia across all age groups were statistically significant. Further well-designed studies are needed in this area, particularly among pediatric patients. Clinical studies to identify the incidence and clinical effects of hyponatremia and hypocalcemia be done. In any critical disease that warrants hospitalization, metabolic derangements may contribute to significant morbidities. It is recommended that derangements be addressed especially in cases where the patients are symptomatic.

REFERENCES

1. Avirutnan P et al. *Dengue virus infection of human endothelial cells leads to chemokine production, complement activation, and apoptosis*. Journal of Immunology, 161:6338—6346; 1998

2. Dengue: guidelines for diagnosis, treatment, prevention and control -- New edition. World Health Organization 2009

3. Department of Health. Disease Surveillance Report Morbidity Week 27, July 1 - 7, 2012

4. Faridi, M, Anju Aggarwal, Manish Kumar, Abedin Sarafrazul.*Clinical and biochemical profile of dengue haemorrhagic fever in children in Delhi*. TROPICAL DOCTOR 2008; 38: 28–30

5. Istúriz R, Gubler D, Brea del Castillo J. *Dengue and dengue haemorrhagic fever in Latin America and the Caribbean*. Infect Dis Clin North Am. 2000;14(1): 121-40

6. Kapoor, S & Ankur Singh. *Hypocalcemic Tetany: An Infrequently Recognized Association with Acute Dengue Infection*. Indian J Pediatr. December 2012. 79(12):1673

7. LumpaopongAdisorn, PinyadaKaewplang, VeerachaiWatanaveeradej, et. al. *Electrolyte Disturbances And Abnormal Urine Analysis In Children With Dengue Infection*. Southeast Asian J Trop Med Public Health. Vol 41 No. 1; January 2010

8. Malavig, G.N., P.K. Ranatunga, S.D. Jayaratne, et al. *Dengue Viral Infections as cause of Encephalopathy*. Indian. J. Med. Microbiol. 2007. 25(2): 143-5

9. Mekmullica J, Suwanphatra A, Thienpaitoon H, et al. *Serum and urine sodium levels in dengue patients*. Southeast Asian J Trop Med Public Health 36: 197-9; 2005

10. Nguyen Thanh Hung, Huan-Yao Lei, Nguyen Trong Lan, Yee-Shin Lin, Kao-Jean Huang, Le Bich Lien, Chiou-Feng Lin, Scott B. Halstead et. al. *Dengue Hemorrhagic Fever in Infants: A Study of Clinical and Cytokine Profiles*. The Journal of Infectious Diseases 2004; 189:221–32

11. Syed, S., et. al. *Elemental profile of blood serum of dengue fever patients from Faisalabad, Pakistan*. International Journal of Chemical and Biochemical Sciences. 6(2014):34-37

12. Varavithya W, Manu P, Kittikool J, Phongbetchara P, Kashemsant C. *Studies on dengue hemorrhagic fever. II: Electrolyte study*. J Med Assoc Thai, 56: 15-23; 1973

13. World Health Organization. Dengue: *guidelines for diagnosis, treatment, prevention and control -- New edition*. World Health Organization; 2009.

COMPARISON OF HEMATOCRIT AND PLATELET LEVELS OBTAINED FROM PERIPHERAL VENOUS CATHETER VS VENIPUNCTURE IN PATIENTS ADMITTED AT PHILIPPINE CHILDREN'S MEDICAL CENTER FOR DENGUE FEVER.

ADRIAN SALVADOR M. DE VERA, MD
FLORENTINA UY-TY, MD

ABSTRACT

OBJECTIVES: To determine the statistical agreement between hematocrit and platelet counts obtained via peripheral venous catheters and venous puncture. It also aims to compare the success rate of obtaining samples via PVC and the hemolysis rate between different gauges of IV catheter.

DESIGN: Prospective Cross-sectional Study

PARTICIPANTS: Clinically stable patients admitted for dengue aged 1 to 18 years old and are eligible to be enrolled in the study. Twenty-two patients were enrolled and completed the study.

INTERVENTION: Paired samples of venous blood collection using venipuncture and PVC on the contralateral arm was done during daily serial CBC monitoring. Hematocrit and platelet values between the two methods were analyzed using Bland-Altman Analysis.

MAIN OUTCOME MEASURES: Statistical agreement of platelet and hematocrit levels obtained using PVC and Venipuncture.

RESULTS: We had 22 patients and a total of 67 paired samples. Out of the 67 samples submitted, two samples each from PVC (2.9%) and venipuncture (2.9%) were clotted. There was100% success rate in both methods at the first attempt of collection. None on the samples had hemolysis. On the average, hematocrit measurements from venipuncture are increased by 1.17 (units) compared to PVC, while platelet measurements from PVC are increased by 4.83 (units) compared to venipuncture. There is no significant difference in both platelet count and hematocrit between the two methods, demonstrating agreement between the two methods.

CONCLUSIONS: There is statistical agreement between samples drawn from PVC and venipuncture in terms of hematocrit and platelet counts in dengue pediatric patients. Success rate and hemolysis rates between the two methods are the same. PVC is an acceptable alternative to venipuncture.

KEYWORDS: Peripheral Venous Catheter, Saline Lock Device, Peripheral Access Device, Dengue Fever, CBC, Hematocrit, Platelet Count

INTRODUCTION

Over the years, dengue fever has been a major public health concern. There has been a dramatic global increase in the frequency of dengue fever, dengue hemorrhagic fever and dengue shock syndrome and their epidemics over the past three decades[1].In fact, in our institution there are 512 patients admitted last 2014 due to dengue. An increase in the number of admitted patients due to dengue was seen last 2015 where 938 patients were admitted.

Part of clinical practice in monitoring patients admitted for dengue is serial determination of complete blood count (CBC) to

asses for laboratory signs of plasma leakage (e.g. thrombocytopenia) and hemoconcentration[1]. The 2011 Dengue Guidelines by the World Health Organization suggests that serial haematocrit should be performed at least every four to six hours in stable cases and should be more frequent in unstable patients or those with suspected bleeding. This means that patients being admitted for dengue fever would be exposed to serial CBC determination via venipuncture or finger-prick. This subjects the patient and their caregivers to emotional and physical distress[2].

One method to minimize such distress is to obtain blood collection from ongoing intravenous infusion sites or Peripheral Vascular Catheter (PVCs), Peripheral Venous Devices(PVDs), Peripheral Access Devices (PADs) or Saline Lock Devices (SLDs). Several studies done in adults have shown that collection of blood from PVCs for selected laboratory tests yield acceptable results when compared with those extracted from venipuncture[3,4,5]. In one study done by Berger-Achituv et. al., published in 2010; they've concluded that PVC sampling is a pain reducing method that can be used for children for selected basic analytes[2]. This however is the first and only study in utilizing PVCs for blood collection in children. Participants of their study are mostly inserted with gauge 22 IV catheters in contrast to the more commonly used gauge 24 and 26 catheters used in our institution. Several studies show that the gauge of IV cannula used is inversely proportional to the hemolysis rate which in turn may affect processing of blood collection[6,7].

To date, there are no studies that have determined the use of peripheral venous catheter in obtaining blood samples for determination of hematocrit and platelet count in children admitted for dengue. This research aims to answer the question "Is PVC blood collection a suitable alternative to venipuncture for hematocrit and platelet count determination in patients with dengue fever?"

This study aims to determine the interchangeability of collecting blood samples from peripheral venous catheter and direct venipuncture for hematocrit and platelet monitoring in patients with dengue. The result of which could potentially lower patient discomfort by reducing rates of venipuncture and finger prick.

Patients admitted in the emergency room and at the wards generally require intravenous access for administration of drugs or IV solutions. It is of common practice that blood collection for several laboratory examinations are collected either via direct venous puncture or finger-prick if applicable. Several studies to date have determined the usability of PVCs for collecting blood specimen. The intention of which is to reduce venipuncture that in turn will increase patient comfort[2]. Most of these studies are done on adult population, one of which was done on pediatric population.

In 2001, John Himberger conducted a study to determine the reliability of using intravenous line in obtaining blood samples. The study involved determination of WBC, RBC, Hemoglobin, Hematocrit, Platelet count, Sodium, Potassium, Glucose, Chloride, Carbon Dioxide, Creatinin and BUN in patients admitted to the emergency room. Thirty patients, ages 18 years and above, were enrolled and analyzed in the study. Using paired T-test and Bland-Altman Limits of Agreement Analysis, their study concluded that there is no significant difference in any the values of the laboratory tests done[8].

To date, there is only one study of similar design conducted on pediatric population. Berger-Achituv et al., conducted a study on 47 patients aged 1 to 16 years. They compared analytes for complete blood count, sodium, potassium, glucose, chloride and urea obtained via venipuncture to that collected through a peripheral venous catheter with ongoing intravenous solution on the contralateral extremity. Data was analyzed using paired t test and Bland-Altman limits of agreement. Their study concluded that except for glucose, PVC sampling was a pain reducing method that can be used for children for selected analytes tested[2]. The study also compared other parameters between the two sampling methods. This included number of attempts needed to collect

blood, time needed to collect the blood, mood of the patient (calm, distressed, crying vigorously) and the rate of hemolysis. There was no significant difference between the number of attempts needed and time to collect blood specimen between the two groups. They have also concluded that patients were more distressed/crying during venipuncture. The rate of hemolysis and success rate of extracting samples were not significantly different between collection method[2].

Several studies however contradict the findings of Berger-Achituv et al. In terms of collection success rate, a study on 73 adults using gauges 18 (n = 6), 20 (n = 69) and 22 (n = 6) showed that increasing catheter size yielded higher success rate of blood collection from peripheral venous catheter[3]. Margo A. Halm did a meta-analysis on 18 studies. Collecting specimen from PVCs may lead in significant hemolysis.Hemolysis is the rupture of red blood cells and can occur in vitro or in vivo. In vitro hemolysis is the leading cause of unsuitable samples referred for routine and urgent laboratory testing[9]. There are several factors that contributed to the increased hemolysis rate, specifically the collapse of the soft plastic IV catheter when negative pressure is applied to collect blood[10]. They have concluded that hemolysis occurred in 3.3 to 77% of blood samples obtained via IV catheters as compared to 0 to 3.8% of that from direct venipuncture thus recommending against the practice of collecting blood from peripheral venous catheter[10]. A similar systematic review was done by Heyeret al. and concluded that the use of venipuncture is associated with reduced hemolysis rates[11].

METHODOLOGY

This is a prospective cross-sectional study. Blood sample were obtained from each patient using an ongoing peripheral vascular catheter and subsequently via venipuncture. Specimens weres ent for complete blood count analysis. Hematocrit and platelet count values were compared between the two methods of obtaining a blood sample. Success rate of blood collection and hemolysis rate were also determined as secondary outcomes of the study. Included were patients aged 1-18 years old admitted at the emergency room or service wards of a tertiary pediatric hospital in the Philippines, and patients diagnosed with dengue fever, including those patients with severe dengue (i.e. Dengue Shock Syndrome) who are clinically stable at the time of blood collection.

A minimum of 65 samples was required for this study considering a 95% confidence level, study power of 80%, and standard difference of 0.72 using the Bland-Altman nomogram. The standard deviations used to compute for the standard difference are 8.46 and 7.30 for infusion and venipuncture, respectively, based from the study of Yazdankhahfard et al* on measuring the hematocrit of 60 patients admitted in hospital using the two blood extraction methods[12].

All patients admitted at the emergency room and service wards of institution from October 1 to 24, 2016 were invited to join the study. A thorough history and physical examination, taking note of the age, gender and diagnosis upon admission, was done on all the patients eligible to be enrolled in the study. The caliber/gauge of IV cannula used, largest possible deemed fit for the patient upon the discretion of the health care professional taking care of the patient, was also recorded.

During each patient's scheduled CBC determination, a blood sample was collected using venipuncture following standard procedure of collection on the extremity contralateral to the PVC site. After which, IV infusion on the PVC was stopped for 30 seconds and a tourniquet was placed proximal to the PVC. Using a 1cc syringe, 1ml of blood was slowly drawn and discarded. Another 0.5ml of blood was then collected slowly using the same method and sent for CBC analysis. The PVC was then flushed with 2ml of normal saline and IV infusion was resumed. The paired PVC and venipuncture extractions were done by the same health care professional.

Blood samples were sent and analyzed for complete blood count using an automated

chemical analyzer (Beckman Coulter Unicell DXH 800). Hematocrit and platelet values were compared between the two methods of obtaining the blood sample. The success rate of blood collection and hemolysis rate (samples that cannot be processed by the machine due to hemolysis) between venipuncture and PVC were compared using paired t test.

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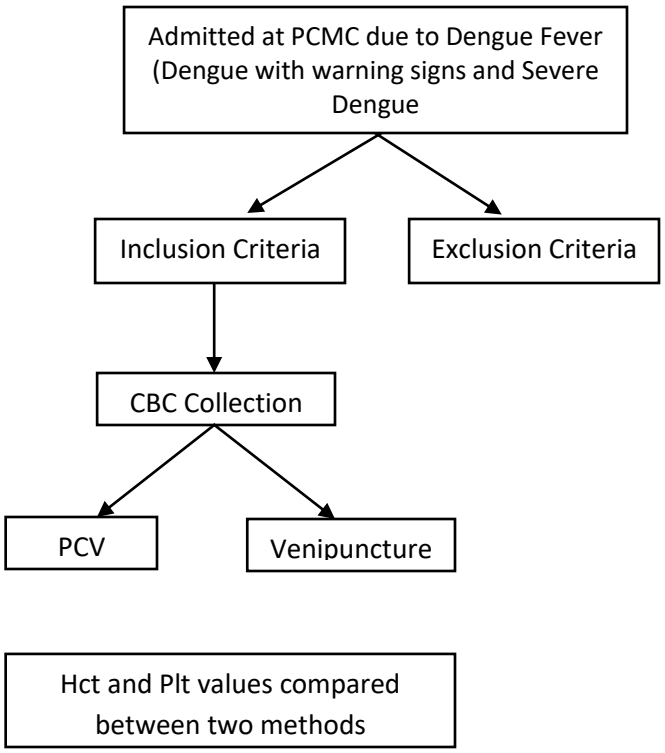


Descriptive statistics was used to summarize the clinical characteristics of the patients. Frequency and proportion was used for nominal variables, median and range for ordinal variables, and mean and SD for interval/ratio variables. Fisher's Exact test was used to determine the difference of proportion of patients on gauge used to occurrence of Hemolysis. To determine the agreement venipuncture and peripheral venous catheter in terms of platelet counts and hematocrit, Bland-Altman analysis was used. Missing variables were neither replaced nor estimated. Null hypotheses were rejected at 0.05α-level of significance. STATA 12.0 was used for data analysis.

RESULTS

A total of 22 dengue patients, with a total of 67 pairs of venipuncture and PVC blood samples, were enrolled to the study. The patients had an average age of 8.98 $\pm$ 4.22 years; 12 were male and 10 were female. Four of the 22 patients had severe dengue, and 18 (82%) had dengue with warning signs. The median number of samples per patient was 2 and ranged from 1 to 7 samples. Gauge 23 was used for venipuncture, and gauges 22 and 24 were used for PVC. All blood extraction attempts were successful on the first attempt. Out of the 67 samples submitted, two samples each from PVC (2.9%) and venipuncture (2.9%) collection were clotted and hence unsuccessfully analyzed by the machine.

Table 1. Demographic and clinical characteristics of dengue fever pediatric patients (n=22 patients, 67 paired samples)

	Frequency (%); Mean ± SD; Median (Range)
Number of children	22
Number of paired blood samples	67
Number of paired samples successfully analyzed by machine	65
Age	8.98 ± 4.22
Sex	
Male	12 (54.55)
Female	10 (45.45)
Diagnosis	
Dengue with warning signs	18 (81.82)
Severe dengue	4 (18.18)
Number of samples per patient	2 (1 to 7)
Venipuncture outcome	
Puncture gauge (23)	67 (100)
Success on first attempt	67 (100)
Clotted sample	2 (2.9)
Peripheral Venous Catheter outcome	
Puncture gauge	
22	6 (27.27)
24	16 (72.73)
IV rate (mL/hr)	60 (30 to 150)
Success on first attempt	67 (100)
Clotted sample	2 (2.9)

To account for the fact that several paired samples arose from repeated measures (n = 61), we computed for the coefficient of repeatability. The coefficient of repeatability for hematocrit is 0.2510 while that for platelet is 1.5265 units. This refers to the minimum change required between repeated measures in order for the paired sample to be included in the analysis (i.e., if in the repeated measures the values did not differ across repeated measures, then we cannot use these values for agreement). For hematocrit, we were able to use 64 pairs, while for platelet, we were able to use 65 pairs.

On average, hematocrit measurements from venipuncture is increased by 1.17 units compared to hematocrit measured by PVC. There is no significant difference in hematocrit between the two methods, demonstrating agreement between the two methods (Table 2, Figure 3).

Table 2. Agreement parameters between Hematocrit from Peripheral Venous Catheter and Venipuncture (n=64)

Limits of Agreement	-5.33 to 7.67
Mean difference of venipuncture hct minus PVC hct (95% CI)	1.17 (0.36 to 1.98)
Range	29.5 to 51
Pitman's Test of difference (r)	-0.199
P-Value	0.129

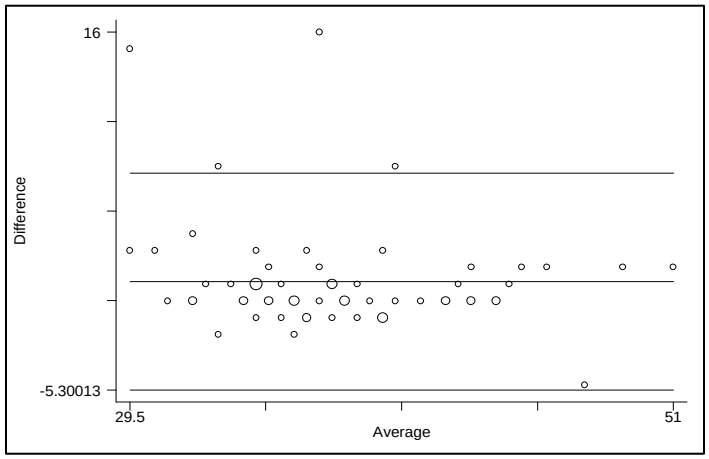


Figure 1. Bland-Altman plot depicting agreement between hematocrit from Peripheral Venous Catheter and Venipuncture

On average, platelet measurements from PVC is increased by 4.83 units compared to platelet count measured by venipuncture. There is no significant difference in platelet count between the two methods, demonstrating agreement between the two methods (Table 3, Figure 4).

Table 3. Agreement parameters between platelet counts from Peripheral Venous Catheter and Venipuncture (n=65)

Limits of Agreement	-51.80 to 42.13
Mean difference between venipuncture plt minus PVC plt (95% CI)	-4.83 (-10.65 to 0.99)
Range	8 to 294.5
Pitman's Test of difference (r)	-0.053
P-Value	0.676

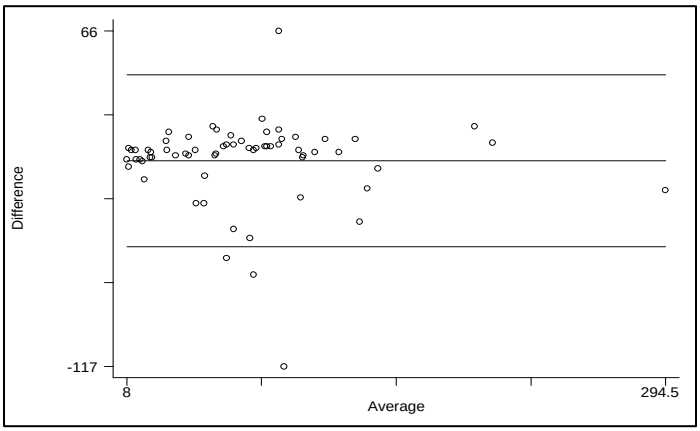


Figure 4. Bland-Altman plot depicting agreement between platelet from Peripheral Venous Catheter and Venipuncture

DISCUSSION

Based on the results presented above, collection of blood for hematocrit and platelet count determination using existing peripheral venous catheters is an acceptable alternative to venipuncture. Results of hematocrit and platelet counts in both methods of collection are agreeable. This is in line with studies already published by Berger-Achituv et al in pediatric population[2] and several studies on adults using various analytes which included CBC determination[3,4,8,5]. This study further increased the sample size of the study on pediatric population and utilized smaller gauge catheters (gauge 22 and 24).

Contrary to a meta-analysis that showed significant hemolysis rates that ranges from 3% to 77% when obtaining specimen from PVCs[10], none of our 67 samples demonstrated hemolysis that would hinder it from being analyzed by our machine. Hemolysis can be caused by several factors; among them are the gauge of catheter used and the pressure while obtaining blood from PVC that causes collapse of the soft IV catheter. Our study encountered zero hemolysis among the 67 samples submitted, this can be explained by the careful aspiration from PVC sites and using only 1cc syringe thus decreasing the pressure that may lead to the collapse of the catheter. Only two samples (2.9%) for both PVC and venipuncture collection (2.9%) were not analyzed by the machine due to clotting.

The study of Katie Prue Owens proved a discard volume of dead space plus 1ml, 2ml or 3ml did not significantly alter results of the analytes tested[5]. Our study further reduced the discard volume to exactly 1ml. No complications were encountered in obtaining blood samples from PVC.

The results of this study will have a positive impact in the management of patients admitted for dengue fever. The amount of venipuncture or finger prick to determine CBC in patients needing serial monitoring can be greatly reduced. This will increase both patients' and relatives' comfort. Health care providers will also benefit from such procedure since collecting blood from existing PVC is an easier procedure, with the same success rate (100% on first attempt), compared to venipuncture.

CONCLUSIONS AND RECOMMENDATIONS

Hematocrit and platelet levels obtained from peripheral venous catheter and venipuncture are comparable. Sufficient quantities of samples were obtained using both gauges 22 and 24 catheters. No hemolysis was encountered. There is a one hundred percent success rate in obtaining samples for both PVC and venipuncture.

The study recommends the use PVC method of collecting specimens for hematocrit and platelet determination in patients admitted for dengue fever. The results from such method agree with traditional venipuncture collection. Furthermore, PVC collection is as easily performed, if not easier, than venipuncture. The greatest benefit of which would be less distress and pain for the patient. Guidelines on collecting specimen using existing PVC should be formulated and incorporated in practice guidelines on managing patients with Dengue Fever.

We recommend conducting similar studies using other analytes (e.g. WBC count, RBC indices and blood chemistries) to be able to fully utilize the value of PVC collection. A higher number of paired samples are also recommended to further strengthen the statistical power of the data collected. Furthermore, a study that does not limit subjects being admitted for dengue is also recommended.

BIBLIOGRAPHY

1. World Health Organization. Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever Revised and expanded edition. India: World Health Organization, Regional Office for South-East Asia; 2011.

2. Berger-Achituv S, Budde-Schwartzman B, Ellis M, Shenkman Z, Erez I. Blood Sampling Through Peripheral Venous Catheters Is Reliable for Selected Basic Analytes in Children. PEDIATRICS. 2010;126(1):e179-e186.

3. Corbo J, Fu L, Silver M, Atallah H, Bijur P. Comparison of Laboratory Values Obtained by Phlebotomy versus Saline Lock Devices. Academic Emergency Medicine. 2007;14(1):23-27.

4. Ortells-Abuye N, Busquets-Puigdevall T, Diaz-Bergara M, Paguina-Marcos M, Sanchez-Perez I. A cross-sectional study to compare two blood collection methods: direct venous puncture and peripheral venous catheter. BMJ Open. 2014;4(2):e004250-e004250.

5. Prue-Owens K. Use of Peripheral Venous Access Devices for Obtaining Blood Samples for Measurement of Activated Partial Thromboplastin Times. Critical Care Nurse. 2006;26(1):30-38.

6. Kennedy C, Angermuller S, King R, Noviello S, Walker J, Warden J et al. A comparison of hemolysis rates using intravenous catheters versus venipuncture tubes for obtaining blood samples. Journal of Emergency Nursing. 1996;22(6):566-569.

7. Dugan L, Leech L, Speroni K, Corriher J. Factors Affecting Hemolysis Rates in Blood Samples Drawn From Newly Placed IV Sites in the Emergency Department. Journal of Emergency Nursing. 2005;31(4):338-345.

8. Himberger J, Himberger L. Accuracy of drawing blood through infusing intravenous lines. Heart & Lung: The Journal of Acute and Critical Care. 2001;30(1):66-73.

9. Lippi G, Avanzini P, Aloe R, Cervellin G. Blood Collection From Intravenous Lines: Is One Drawing Site Better Than Others?. Lab Med. 2014;45(2):172-175.

10. Halm M, Gleaves M. Obtaining Blood Samples From Peripheral Intravenous Catheters: Best Practice?. American Journal of Critical Care. 2009;18(5):474-478.

11. Heyer N, Derzon J, Winges L, Shaw C, Mass D, Snyder S et al. Effectiveness of practices to reduce blood sample hemolysis in EDs: A laboratory medicine best practices systematic review and meta-analysis. Clinical Biochemistry. 2012;45(13-14):1012-1032.

12. Yazdankhahfard MR, Taghizadeganzadeh M, Reza M, Farzaneh, Mirzaei K. Comparison of complete blood count of blood samples taken through venipuncture and through peripheral venous infusion line after the administration of fluids. *Pars Journal of Medical Sciences* 2015; 12(4): http://jmj.jums.ac.ir/files/site1/user_files_204932/eng/2471777708-A-10-379-1-13c0786.pdf (accessed 27 January 2016)

A COMPARATIVE STUDY OF ACETATED ISOTONIC ELECTROLYTE SOLUTION, NORMAL SALINE SOLUTION, AND LACTATED RINGER'S SOLUTION IN THE INITIAL FLUID RESUSCITATION OF CHILDREN 1 MONTH TO 18 YEARS OLD WITH SEVERE DENGUE AT THE PHILIPPINE CHILDREN'S MEDICAL CENTER

ALLEN KILBY M. PALON, MD
MARY JOY S. TORRES, MD, ERVINA J. ASTIH, MD
MELLINOR A. ASPURIA-ANG, MD

ABSTRACT

BACKGROUND: At PCMC, acetated isotonic electrolyte solution is used in the initial resuscitation in severe dengue patients. However, no local study has comparedacetated isotonic electrolyte solution against normal saline and lactated Ringer's solutions.

OBJECTIVE: This study aims to determine the comparative recovery time to achieve initial and sustained cardiovascular stability in severe dengue patients using acetated isotonic electrolyte solution, normal saline solution, and lactated Ringer's solution.

METHODOLOGY: This is a retrospective cohort study involving 166 severe dengue patients 1 month to 18 years old admitted at the PICU from 2014 to 2016. They were divided into 3 groups based on the initial fluid used: 58 in the AIES group, 58 in the NSS group, and 50 in the LRS group.

RESULTS: AIES group had the shortest time to achieve initial and sustained stability among patients without re-shock. Also, AIES group needed less fluid to establish stability and had less re-shock, less use of colloid and inotropes, less blood transfusion,and less need for mechanical ventilation and dialysis. NSS had the most fluid shift to AIES and/or colloid while LRS had the most colloid used. Hyperchloremic metabolic acidosis was mostly seen in the NSS group. The length of ICU stay was almost the same in all groups. There was zero mortality in AIES group as compared to 3 on NSS group and 2 in LRS group.

CONCLUSION AND RECOMMENDATION: Acetated isotonic electrolyte solution is more effectivethan normal saline and lactated Ringer's solutions in initial fluid resuscitation among severe dengue patients. It should be the fluid of choice in the initial resuscitation among severe dengue patients. It is recommended that a randomized control study with more patients be conducted.

KEYWORDS: dengue, acetated isotonic electrolyte solution, normal saline, lactated Ringer's, resuscitation

INTRODUCTION

Dengue continues to be a health problem in the Philippines. The first dengue epidemic in Manila dates back in 1954.[1] From then on, the number of dengue cases increases every year. According to the 2010 World Health Organization's (WHO) annual dengue data report, the Philippines had the highest dengue cases (135,355 cases) and deaths (0.6%) in the Western Pacific Region.[2] But with the updates in the management of dengue in the region, the mortality rate has a decreasing trend.[2,3]

Local data from the Department of Health (DOH) showed a total of 101,401 suspected dengue cases nationwide from January to August 20, 2016. This number is 16% higher compared to 2015. Most of the cases were from the following regions: Region VI (11.1%), Region IV-A (10.7 %), Region VII (9.8%), Region X (8.8%) and Region XII (8.6%). The ages of these patients ranged from less than 1 month to 100 years old, with median of 13 years old, with more male patients at 52.4%. In addition to that, most of the cases belonged to the pediatric age group of 5 to 14 years old (38.8%).

Fluid management in dengue depends on its severity based on the WHO guideline, which was later adopted by the DOH. Based on the 2012 dengue guideline of the Philippine Pediatric Society (PPS) and the DOH, dengue is now classified as dengue with or without warning signs and severe dengue.[4] According to this guideline, crystalloids such as normal saline and lactated Ringer's solution, and colloids such as Dextran, Voluven, Tetraspan, and Gelatin, can be used as the first line in fluid resuscitation in severe dengue as these fluids have shown its effectiveness in reducing the recurrence of shock and mortality.[4] However, each has its own risks and benefits. Compared with crystalloids, colloids are more expensive and have higher risk for allergic reactions.[4] Because of these, several studies have been conducted comparing normal saline, lactated Ringer's solution and colloids. But there are still insufficient data on the advantage of one fluid over the other when it comes to fluid resuscitation in cases of severe dengue. In addition to that, there are also limited studies conducted using the balanced crystalloids these days. No extensive studies have been conducted comparing the use and effects of balanced crystalloid such as acetated Ringer's solution (isotonic electrolyte solution) to that of another balanced crystalloid such as lactated Ringer's solution, or isotonic electrolyte solution to that of normal saline and colloid.

Normal saline (0.9% NaCl solution) is the most used fluid not just in dengue cases for resuscitation or maintenance solution since it has shown that its use is equivalent or at times more superior than colloid solutions.[5] It is composed of 154 milliequivalent of sodium and 154 milliequivalent of chloride per 1000mL of the solution. On the other hand, colloid solutions, categorized as blood-derived and semi-synthetic, consist of large molecules combined in a base solution such as normal and hypertonic saline, and others. The main reason for its use is to expand the intravascular volume longer and effectively.[6]

Balanced crystalloid solutions are developed as it closely mimics the ionic composition of the blood. These solutions include the lactated Ringer's solution with lactate as the buffer and isotonic electrolyte solution with acetate and malate as the buffer. These solutions contain less sodium and chloride content as compared to that of normal saline and colloid solutions.

These ionic components, as well as the buffer of the balanced solutions, have varying effects on the acid-base balance, renal function, electrolyte level, and vascular permeability.[5] Hence, in severe dengue, which has severe plasma leakage, severe active bleeding and severe organ involvement, the choice of fluid, especially during the early resuscitation, plays a very important role in the management and eventual outcome in such cases. Hence, our aim was to determine the comparative recovery time to achieve sustained cardiovascular stability among severe dengue patients using acetated isotonic electrolyte solution, normal saline solution, and lactated Ringer's solution.

According to the PPS revised dengue guidelines released in 2012[4], the fluid of choice when it comes to severe dengue with compensated shock is crystalloid given for an hour, and if with hypotensive shock, crystalloid or colloid may be used as the first line of intravenous fluids, which is given for 15 minutes. Reassessment after fluid resuscitation is done and the next intravenous fluid to be used depends if there is improvement of the blood pressure, heart rate, respiratory rate, peripheral perfusion, capillary refill time, and sensorium. In addition to that, it has been stated in this guideline that normal saline can cause

hyperchloremic metabolic acidosis, which can be mistaken as lactic acidosis from prolonged shock. On the other hand, Ringer's lactate solution should be avoided in patients with liver failure and colloids may cause coagulopathy but none yet was seen in dengue patients.

In 2003, a local study done in our institution by Cifra and Nazareno compared the effectiveness of a colloid (6% Haes-Steril) and crystalloid (Ringer's lactate) as the first-line plasma substitute for the management of DSS.[9] Twenty-seven patients were included in this study. The colloid group had longer duration of control of shock as compared to the crystalloid group. Also, there was less recurrence of shock in the colloid group. Both groups have the same average hospital stay. However, when compared to the crystalloid group, the colloid group had a lower mortality rate when used initially.

In 2010, a local study by Jalac et al reviewed the use of colloids and crystalloids in DSS among pediatric patients.[12] They did a systematic review and meta-analysis of both fluids comparing their effects in reducing the recurrence of shock, the need for rescue fluids, the need for diuretics, total volume of fluids infused, the hematocrit levels and pulse rates, and the mortality rates among these patients. They have found out that both colloids and crystalloids did not differ significantly in terms of decreasing the recurrence of shock, and the need for rescue fluids and diuretics. However, they have found out that there was an improvement of the hematocrit level and the pulse rates in the colloid group from the baseline after two hours of fluid resuscitation.

Also in 2010, Akech et al also did a review comparing the effects of using crystalloids and colloids in fluid resuscitation among children of aged 1 month to 12 years old with severe infection.[13] A total of nine trials were included in the review but only eight trials compared the use of crystalloid and colloid. They found that compared with crystalloids, a weak evidence that using colloids for volume expansion could have a better survival in children with certain severe infections.

In 2013, Perel did a review of randomized control trials comparing the effect of crystalloid and colloid in fluid resuscitation in patients with dengue fever.[16] Five trials were included in the review on the effects of colloids versus crystalloids. Three studies compared dextran (colloid) with crystalloids and two studies compared gelatins with crystalloids. He found out that there is no evidence that fluid resuscitation with colloids reduced the mortality as compared to crystalloids. However, because of the low quality of evidence included in his review, as outcomes other than mortality were included in the review, colloids should not be included for volume replacement in dengue patients.

In a review done by Córtes et al in 2014, they determined the advantages and disadvantages of using different isotonic crystalloid in surgical and non-surgical adult patients.[18] They have found out that the use of normal saline increased the incidence of hyperchloremic acidosis, which could have an adverse effect on inflammation and coagulation. Ringer's lactate increased the blood lactate levels when used in large amounts. It was also reported that hyperchloremic acidosis might cause renal vasoconstriction, however, its effect on renal function, needed more studies. Aside from that, the use of normal saline appeared to be associated with increased blood loss hence requiring more transfusions as compared to those patients in which Ringer's lactate were used. However, there was a clear association of lactate levels with mortality when large volume of Ringer's lactate was used. But in contrast, lactate may be used as a fuel for the myocardium, neurons, and red blood cells among others.

Another review of randomized trials done by McDermid et al in 2014 discussed the controversies of type, dose, and toxicity of different fluids used in acutely ill hospitalized patients.[19] When crystalloid was compared against colloid, differences in efficacy were modest but differences in safety were significant.

Moreover, differences in chloride load and strong ion difference across solutions appeared to be clinically important as a high chloride load could cause metabolic acidosis. Quantitative toxicity (fluid overload) was associated with adverse outcomes and could be diminished with fluid therapy based on functional hemodynamic parameters. Qualitative toxicity (fluid type), in particular for iatrogenic acute kidney injury and metabolic acidosis, remained to be a concern for synthetic colloids and isotonic saline, respectively. Because of these, physiologically balanced crystalloids may be utilized, as the fluid of choice for acutely ill patients.

In 2015, Varrier and Ostermann did a review of different fluids and their composition and its effect on acid-base status, renal function, and coagulation, hence affecting outcomes.[22] They reviewed three fluids – unbalanced crystalloids (normal saline, dextrose solutions, sodium bicarbonate solutions), balanced crystalloids (Ringer's lactate, Plasma-Lyte, Sterofundin), and colloids (albumin, gelatin, dextran, starch). In terms of volume expansion, the effects of fluid infused depend on the molecular weight and half-life of the molecular contents of the fluids, endothelial integrity, as well as hydrostatic and osmotic pressure gradient between the intravascular and extravascular compartment. In cases of acute volume loss or redistribution, the low hydrostatic pressure could cause prolonged filling effect of both crystalloids and colloids. Therefore, the benefit of both crystalloids and colloids were the same. Hyperchloremia and metabolic acidosis were more commonly seen in normal saline causing renal vasoconstriction, and in effect reduced glomerular filtration. When compared to Ringer's acetate, Ringer's lactate and normal saline, hydroxyethyl starches have shown an increased need for renal replacement therapy in patients with renal dysfunction. Negative effects on hemostasis were seen more in colloids. With this review, it preferred to use balanced crystalloid to normal saline to minimize fluid overload, and the avoidance of synthetic colloids.

METHODOLOGY

This is a retrospective cohort study that included the charts of all patients aged 1 month to 18 years of age with a diagnosis of severe dengue (DSS III and IV) at the emergency room and subsequently admitted to the intensive care unit of our institution from 2014 to 2016. The groups were divided based on the fluid of choice during the initial fluid resuscitation – (A) acetated isotonic electrolyte solution, (B) normal saline solution, and (C) lactated Ringer's solution. The age and sex in each group were recorded as well as the time of initial cardiovascular stability and sustained cardiovascular stability. Their physical examination findings (pulse pressure, heart rate) and urine output were also recorded. Laboratory findings – hematocrit level, platelet count, electrolytes level (sodium, potassium, chloride, calcium), lactate, venous blood gases (pH, pCO_2, HCO_3), alanine and aspartate transaminases, blood urea nitrogen, and creatinine – were recorded and assessed accordingly. The PELOD-2 Score was computed for each patient. The total volume of each intravenous fluid used per patient was also recorded accordingly. Rescue colloid was also recorded in groups A, B, and C, if given. The outcome after the first two hours of fluid resuscitation, the length of ICU stay and the need for renal replacement therapy, mechanical ventilation, and mortality rate were recorded in each group.

All the records of those patients who satisfied the inclusion criterion were reviewed and analyzed accordingly. All the fluids used, the volume of each fluid given, age, sex, blood pressure, heart rate, urine output, hematocrit level, white blood cell count, platelet count, serum sodium, potassium, chloride, calcium, and lactate levels, pH, pCO2, and HCO_3 levels, transaminases, blood urea nitrogen, creatinine, Glasgow Coma Scale, pupillary reaction, time of initial and sustained cardiovascular stability, presence of re-shock, need for colloids, inotropes, and blood transfusion, use of renal replacement therapy and mechanical ventilation, length of stay in the intensive care unit, and the

mortality rate were all recorded, reviewed, and analyzed accordingly.

The primary outcome was the time to achieve sustained cardiovascular stability from initial fluid resuscitation among severe dengue patients. Secondary outcomes were: time to achieve initial cardiovascular stability from the time of recognition of shock and re-shock; time to achieve sustained cardiovascular stability from re-shock; total volume of fluid to achieve cardiovascular stability; pattern of change in hematocrit level; presence of electrolyte imbalances and acid base disturbances before and after treatment, and their correlation with anion gap and strong ion difference; presence of deranged transaminases before and after treatment; PELOD-2 Score and the probability of mortality based on the initial white blood cell count and platelet count, Glasgow Coma Scale, pupillary reaction, serum lactate, mean airway pressure, serum creatinine, PaO_2, $PaCO_2$, and the use of mechanical ventilation; re-shock time and rate; use of other intravenous fluids, inotropes, and blood transfusions; need for dialysis; length of stay at the pediatric intensive care unit; and mortality rate in each group.

Analysis of variance and chi-square test were used to evaluate the association between the clinical and laboratory parameters, and the primary and secondary outcomes. A 95% confidence interval was set to measure the association among outcome measures and a p-value of less than 0.05 was considered significant. To eliminate heterogeneity and to test those statistically significant variables, multivariate regression analysis was used.

RESULTS

A total of 175 patients were admitted at the PICU with severe dengue from January 2014 to December 2016; 166 of these were included in the study and divided into 3 IVF groups:58 patients were included in the AIES group (acetated isotonic electrolyte solution); 58 patients were included in the NSS group (normal saline solution); and 50 patients in the LRS group (lactated Ringer's solution). Nine patients were excluded because of the presence of co-morbidities (rheumatic heart disease, pulmonary tuberculosis, and red cell aplasia), transfusion of packed red blood cell at the level of the emergency room, received fluid resuscitation from other institution and transferred to our institution, those patients who were initially admitted at the wards as dengue with warning signs, and in which colloid was initially used for fluid resuscitation.

The demographics of the patients and initial physical examination are summarized in Table 1. All three groups have the same gender distribution. NSS group had the youngest patients with mean age of 7.54 years while LRS group had the oldest patients at 9.3 years old, and AIES group at 8.0 years old. LRS group had the heaviest patients with mean weight of 30.7kgs as compared to 24.88kgs for the AIES group and 25.66kgs for the NSS group. Pulse pressure among the three groups was further divided according to age and was classified as (1) pulse pressure not detectable, or less than or equal to 20mmHg, and (2) pulse pressure between 21 – 40mmHg. Sixty-eight patients between 1 month old and 8 years of age have pulse pressure which were non-detectable or equal or less than 20mmHg. This finding was consistent among patients who were 9 – 18 years old, with a total of 52 patients. As a compensation for the shock, ninety-nine patients showed tachycardia, with a mean heart rate of 108 – 115 beats per minute among the three IVF groups. Further dividing the heart rate among age groups showed that 63 patients developed tachycardia among1 month to 8 years old as compared to 36 patients among patients who were 9 – 18 years old. Initial measurement of the urine output was adequate with a mean of 1.34mL/kg/hour for all the three IVF groups.

Table 1. Summary of the Demographics and Initial Physical Examination

	Acetated Isotonic Electrolyte Solution (n = 58)	Normal Saline Solution (n = 58)	Lactated Ringer's Solution (n = 50)	p value
Gender				
• **Male**	34	25	24	0.2337
• **Female**	24	33	26	
Age, years				
• **Mean ± SD**	8 ± 3.6	7.5 ± 3.9	9.2 ± 3.3	
• **1month to 8years old**	36	38	20	**0.0438**
• **9 – 18 years old**	22	20	30	
Body Weight, kg				
• **Mean ± SD**	24.88 ± 9.81	25.66 ± 10.99	30.68 ± 12.13	**0.01459**
Heart Rate, bpm				
• **Mean ± SD**	114.43 ± 20.62	114.63 ± 18.99	108.54 ± 23.06	
• **1 month to 8 years old**				
○ **Normal**	9	13	6	
○ **High**	26	23	14	0.23881
• **9 – 18 years old**				
○ **Normal**	12	9	18	
○ **High**	11	13	12	
Pulse Pressure, mmHg				
• **Mean ± SD**	24.13 ± 16.33	31.72 ± 22.49	25.8 ± 15.53	
• **1 month to 8 years old**				
○ **Non-detectable, palpatory, =<20mmHg**	27 (47%)	26 (45%)	15 (30%)	
○ **21 – 40mmHg**	9 (16%)	12 (21%)	5 (10%)	0.07250
• **9 – 18 years old**				
○ **Non-detectable, palpatory, =<20mmHg**	19 (32%)	13 (22%)	20 (40%)	
○ **21 – 40mmHg**	3 (5%)	7 (12%)	10 (20%)	
Urine Output, mL/kg/hour				
• **Mean ± SD**	1.22 ± 0.68	1.35 ± 0.75	1.46 ± 0.71	0.23121

Initial hematocrit level was determined prior to fluid resuscitation and repeat hematocrit level determination was done on the 4th, 8th, and 12th hour after fluid resuscitation as shown in Table 2. The levels of hematocrit on the 4th, 8th, and 12th hour after fluid resuscitation is shown on Figure 1. The mean initial hematocrit level in the AIES group was at 45.6, 44.5 in the NSS group, and 44.3 in the LRS group. There were 36 patients in the AIES group, 29 patients in the NSS group, and 24 patients in the LRS group who have hemoconcentration. Regardless of the IVF groups, more patients presented with hemoconcentration – 54 patients from the1 month to 8 years old age group as compared to 35 patients from the 9 – 18 years old age group. After the 4th, 8th, and 12th hour of fluid resuscitation, the hematocrit level showed a decreasing trend among the three IVF groups. The mean hematocrit level was that of 42.02among patients in the AIES group, 40.18 among patients in the NSS group, and 40.24

among patients in the LRS group after 12 hours of fluid resuscitation. This was translated to 7.85%, 9.71%, and 9.21% decrease from the initial hematocrit level among the AIES, NSS, and LRS groups, respectively, thus showing that the AIES group had the modest decrease of hematocrit levels that did not translate to bleeding, hemodilution, and/or the need for blood transfusion.

Figure 1. Pattern of Decrease of Hematocrit Level from Initial Determination

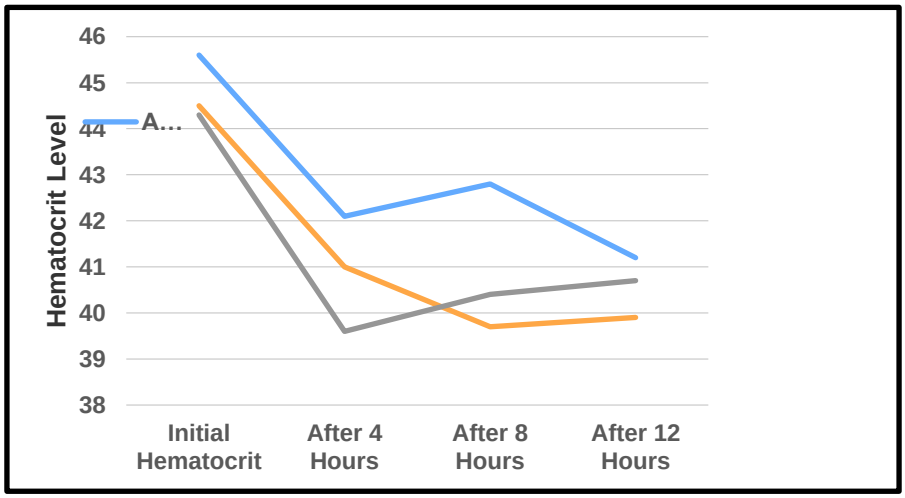


Table 2. Hematocrit Levels onInitial Presentation, and After 4, 8, and 12 Hours of Fluid Resuscitation

	Acetated Isotonic Electrolyte Solution (n = 58)	Normal Saline Solution (n = 58)	Lactated Ringer's Solution (n = 50)	p value
Hematocrit, Initial				
• **Mean ± SD**	45.6 ± 6.20	44.5 ± 5.81	44.32 ± 4.81	0.43482
• **1 month to 8 years old**				0.94904
○ **Low**	1	1	1	
○ **Normal**	13	16	8	
○ **High**	22	21	11	
• **9 – 18 years old**				
○ **Low**	1	0	0	
○ **Normal**	7	12	17	
○ **High**	14	8	13	
Hematocrit, after 4 hours				
• **Mean ± SD**	42.06 ± 4.81	40.97 ± 5.94	39.62 ± 5.44	0.10404
Hematocrit, after 8 hours				
• **Mean ± SD**	42.78 ± 4.78	39.69 ± 3.37	40.38 ± 4.57	**0.00678**
Hematocrit, after 12 hours				
• **Mean ± SD**	41.21 ± 5.01	39.87 ± 4.62	40.72 ± 5.92	0.38770
Hematocrit from 4 – 12 hours				
• **Mean ± SD**	42.02 ± 4.89	40.18 ± 4.84	40.24 ± 5.39	0.83982
• **% Decrease from Initial Hematocrit**	7.85	9.71	9.21	

Hyponatremia and hypocalcemia were noted in the initial serum electrolytes determination. Only 45 patients in the AIES group did a repeat serum electrolyte level determination as compared to 50 patients in the NSS group and 38 patients in the LRS group. Repeat serum sodium in the AIES and LRS group showed minimal effect on serum sodium level as it remained to be low which can be attributed to active plasma leakage due to increased permeability of the blood vessels in dengue infection. There was no incidence of hypernatremia in the AIES group while there was a decrease by 1.34-times (34% decrease) in the number of patients with hypernatremia in the LRS group. On the NSS group, 54% still have normal serum sodium level but there was a2.9-times increase (190% increase)in the number of patients who developed hypernatremia. Repeat serum potassium levels were maintained normal but there was persistence of hypocalcemia on the three IVF groups.

Most patients in the AIES group have a repeat serum chloride levels which were normal, but it is important to note that there was an increase by 1.3-times (29% increase) in the number of patients who developed hyperchloremia. On the other hand, the LRS group also had a normal repeat serum chloride levels in most of the patients but there was an increase by 3.9-times (294% increase) in the number of patients who developed hyperchloremia. This is comparable to the NSS group as there was an increase by 3.8-times (277% increase) in the number of patients who developed hyperchloremia.

Same initial serum pH levels were noted among the three IVF groups. The AIES group showed marked reversal of metabolic acidosis as compared to the NSS and LRS groups as evident on the serum pH at the 4^{th} to 12^{th} hours after fluid resuscitation. This normalization of the serum pH was more consistent with the increase in serum HCO_3 in the AIES group as compared to the NSS and LRS groups, of which the HCO_3 levels remained to be low. The changes in serum pH and HCO3 are shown in Figures 2 and 3, respectively.

Different references have different normal values of anion gap and strong ion difference. In this study, a normal anion gap has a range of 5 – 20mmol/L and a normal strong ion difference is between 38 to 46mmol/L. Anion gap in all groups were normal even on repeat determination after the 12^{th} hour of fluid resuscitation as shown in Table 3. However, there was a trend of closure of the anion gap in the AIES and LRS groups as compared to the NSS group, which remained to be on the high-normal side. The initial strong ion difference in the AIES and LRS group were normal as compared to the low strong ion difference in the NSS group. However, all the three IVF groups have lower strong ion difference after the 12^{th} hour of fluid resuscitation.

The presence of a buffer system in AIES and LRS produces more bicarbonate to correct the acidosis as compared to NSS which do not have a buffer system. But due to the presence of hepatic insult, lactate from LRS is converted to lactic acid instead of bicarbonate, thus, producing more acidosis. This can explain why the LRS group remained to have metabolic acidosis despite the presence of buffer systems. Moreover, NSS contains higher chloride content as compared to AIES and LRS. This high chloride content of the NSS can also contribute in the development of metabolic acidosis. In hyperchloremic metabolic acidosis, there is a normal anion gap and low strong ion difference. Hence, hyperchloremic metabolic acidosis can be seen mostly in the NSS group as most patients presented with hyperchloremia with associated normal anion gap metabolic acidosis and low strong ion difference.

Table 3. Change in Serum pH, HCO3, Anion Gap, and Strong Ion Difference Among the AIES, NSS, and LRS Groups

	Acetated Isotonic Electrolyte Solution (Mean ± SD)	Normal Saline Solution (Mean ± SD)	Lactated Ringer's Solution (Mean ± SD)	p value
pH				
• Initial	7.34 ± 0.07	7.33 ± 0.07	7.36 ± 0.07	0.32669
• After 4 Hours	7.36 ± 0.05	7.32 ± 0.09	7.38 ± 0.70	0.07336
• After 8 Hours	7.28 ± 0.084	7.36 ± 0.10	7.35 ± 0.88	0.24513
• After 12 Hours	7.41 ± 0.057	7.36 ± 0.073	7.32 ± 0.10	0.10164
HCO_3				
• Initial	15.38 ± 4.78	14.69 ± 3.48	17.68 ± 6.17	**0.00522**
• After 4 Hours	16.04 ± 3.35	15.20 ± 4.06	19.08 ± 7.06	0.08408
• After 8 Hours	13.35 ± 5.02	16.80 ± 3.33	20.31 ± 9.06	0.07722
• After 12 Hours	18.30 ± 3.83	16.36 ± 3.55	16.37 ± 7.34	0.66395
Anion Gap				
• Initial	18.69 ± 5.05	17.89 ± 14.23	18.97 ± 6.22	0.82540
• Repeat	14.95 ± 3.91	18.27 ± 4.80	13.51 ± 7.24	0.05012
Strong Ion Difference				
• Initial	38.39 ± 4.34	36.71 ± 13.75	40.72 ± 4.44	0.06715
• Repeat	36.48 ± 3.76	35.31 ± 4.94	37.36 ± 3.73	0.07843

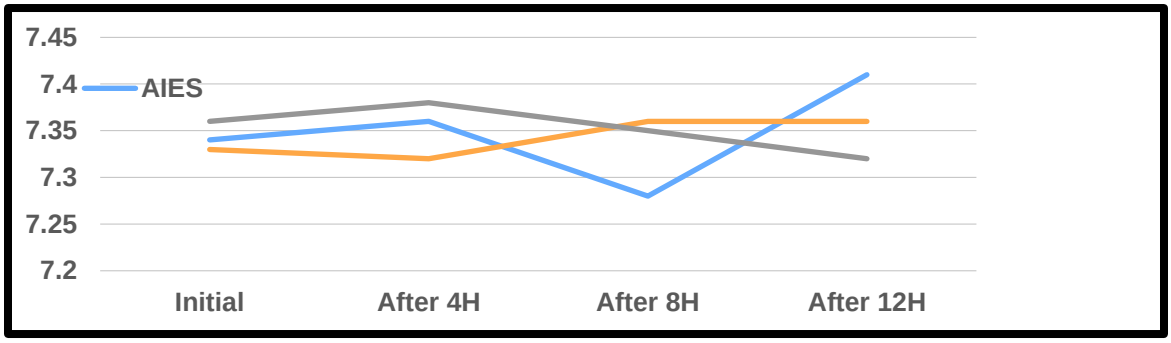


Figure 2. Change in Serum pH in the AIES, NSS, and LRS Groups

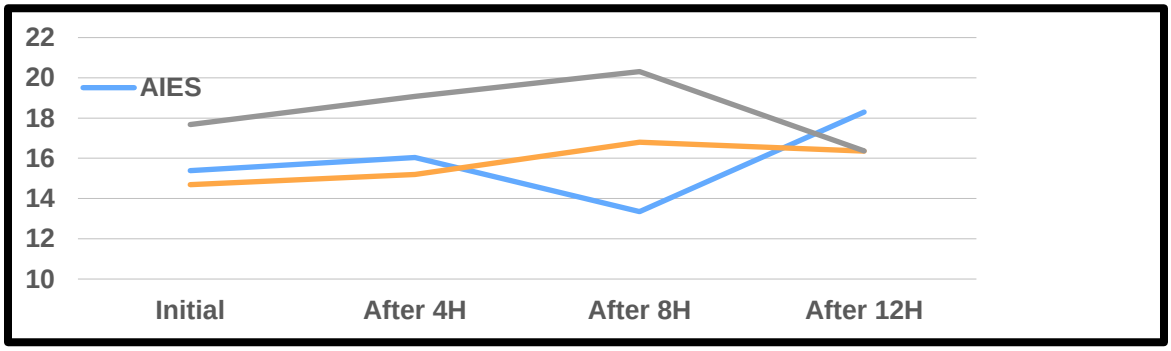


Figure 3. Change in Serum HCO_3 in the AIES, NSS, and LRS Groups

All patients in the three IVF groups have elevated liver transaminases. However, by following the WHO criteria for elevated liver enzymes, only 4patients in the AIES group have elevated liver enzymes of more than 1000mmol/L – 1patient had elevated alanine transaminase and 3patients have elevated aspartate transaminase. Out of these 4 patients, only 1 patient did a repeat transaminase level and although the level was still elevated based on normal range, it showed a 68% decrease from the initial aspartate transaminase level.

Moreover, 42 patients in the NSS group have elevated serum aspartate transaminase levels but only 2 out of these 42 patients have more than 1000mmol/L. These 2 patients were also included in the 8 patients who have repeat serum transaminase determination. Like the AIES group, these patients still have elevated serum aspartate levels but there was a decreasing trend.

The LRS group have a total of 34 patients with elevated transaminase levels but only 2 patients have levels more than 1000mmol/L, one of which eventually died. Only 3 patients did a repeat determination of the transaminase levels and like the AIES and NSS groups, the levels showed a decreasing trend.

Normally, the buffer systems of the AIES and LRS produce more bicarbonate with the conversion of the acetate and malate from the AIES and lactate from the LRS. Acetate and malate are metabolized primarily in the muscle as compared to lactate which is metabolized mainly in the liver. In the presence of hepatic injury, the lactate in the LRS produces more lactic acid instead of bicarbonate, rendering the serum pH more acidotic as seen in the repeat pH determination among the three groups.

N-acetylcysteine is commonly used for paracetamol toxicity. Its role in dengue-associated hepatitis is not yet well-studied. In this study, all patients with serum transaminase levels of more than 1000mmol/L were given N-acetylcysteine. However, it is important to note that only half of the patients in the AIES group with elevated transaminase levels was given N-acetycysteine as compared to 62% in the NSS group and 42% in the LRS group. Although still elevated, there was a decreasing trend on repeat determination of serum transaminase levels in those patients that were given N-acetylcysteine as shown in Figure 4.

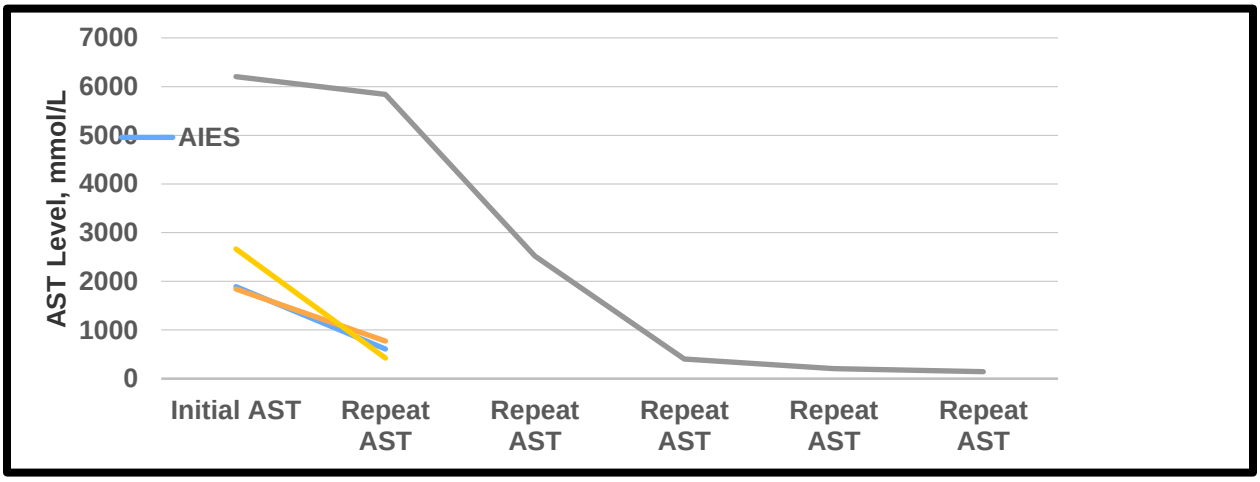


Figure 4. Pattern of Decrease of Serum Aspartate Transaminase Levels

Half of the patients in the three IVF groups have a low initial BUN levels and most have normal initial creatinine levels. The trend of the BUN and creatinine levels is shown on Figures 5 and 6, respectively. In the AIES group, only 1 patient had repeat determination of BUN and creatinine, however, both remained to be normal. In the NSS group, 3 patients have repeat BUN and creatinine levels – 2 patients remained to have normal repeat BUN determination and

with normalization of the creatinine levels; the other 1 patient who initially presented with an elevation of both BUN and creatinine still have further increase of both BUN and creatinine levels which warranted renal replacement therapy. In the LRS group, only 3 patients have repeat determination of BUN and creatinine levels – 2 patients have an increasing trend, of which 1 underwent renal replacement therapy, however, both eventually died; the other 1 patient have BUN level which was both normal on initial and repeat determination, and although there was a 28% increase in the creatinine level, this patient did not require renal replacement therapy.

Table 4 shows that the AIES group had the shortest time to achieve initial stability at 2.29 hours among those patients without re-shock after the initial fluid resuscitation as compared to 2.5 hours in the NSS group and 2.68 hours in the LRS group. This was consistent with the shortest time of 4.37 hours to achieve sustained cardiovascular stability in the AIES group as compared to4.63 hours in the NSS group and 4.94 hours in the LRS group.

Table 4. Summary of Time to Achieve Initial and Sustained Cardiovascular Stability

	Acetated Isotonic Electrolyte Solution (n = 58)	**Normal Saline Solution (n = 58)**	**Lactated Ringer's Solution (n = 50)**	**p value**
Time to Achieve Initial Stability from Initial Shock, hours				
• Mean ± SD	2.29 ± 0.59	2.5 ± 0.68	2.68 ± 0.97	**0.03148**
Time to Achieve Sustained Stability from Initial Shock, hours				
• Mean ± SD	4.37 ± 0.53	4.63 ± 0.94	4.94 ± 1.06	**0.01192**
Time to Achieve Initial Stability from Re-Shock, hours				
• Mean ± SD	2.3 ± 0.48	2.18 ± 0.40	2.35 ± 0.74	0.70603
Time to Achieve Sustained Stability from Re-shock, hours				
• Mean ± SD	4.2 ± 0.42	4.06 ± 0.59	5.33 ± 3.52	0.25341

Moreover, the AIES group had less tendencies to have re-shock at 17% versus 28% for the LRS group and 29% for the NSS group. However, in patients who have re-shock, the NSS group had the shortest time to achieve initial and sustained cardiovascular stability at 2.2 hours and 4.1 hours, respectively, as compared to 2.3 hours and 4.2 hours for the AIES group, and 2.4 hours and 5.3 hours for the LRS group.

The AIES group needed less fluid to establish initial cardiovascular stability at 0.84L as compared to 1.1L for the LRS group and 1.0L for the NSS group as shown in Table 5. Likewise, the AIES group needed less fluid to achieve sustained cardiovascular stability at 1.3L as compared to 2.0L for the LRS group and 1.9L for the NSS group. Since the LRS group had the heaviest mean body weight among the three IVF groups and had a longer time to achieve initial and sustained cardiovascular stability, it had the least amount of fluid at 13.22mL/kg and 10.09mL/kg to achieve initial and sustained cardiovascular stability, respectively, as compared to 15.12mL/kg and 10.51mL/kg for the AIES group and 15.72mL/kg and 10.98mL/kg for the NSS group.

Table 5. Total Volume to Achieve Initial and Sustained Cardiovascular Stability

	Acetated Isotonic Electrolyte Solution (n = 58)	Normal Saline Solution (n = 58)	Lactated Ringer's Solution (n = 50)	p value
Total Volume of IVFto Achieve Initial Stability, mL				
• Mean ± SD	839.66 ± 478.28	993.79 ± 614.86	1090.66 ± 580.03	0.06663
Total Volume per Body Weightto Achieve Initial Stability, mL/kg				
• Mean ± SD	15.12 ± 4.67	15.72 ± 5.63	13.22 ± 2.98	**0.01705**
Total Volume of IVFto Achieve Sustained Stability, mL				
• Mean ± SD	1326.43 ± 618	1922.29 ± 2285	2045.74 ± 1726	0.05856
Total Volume per Body Weightto Achieve Sustained Stability, mL/kg				
• Mean ± SD	10.51 ± 2.53	10.98 ± 3.48	10.09 ± 1.87	0.25032

Table 6 summarizes the differences between the three IVF groups on the use of another crystalloid and/or colloid, transfusion of blood, use of inotropes, and mechanical ventilation. All three IVF groups required the use of colloids especially in episodes of re-shocks. Among the three IVF groups, the LRS group had more tendencies of using colloid at 34%, hence, the LRS group did not necessitate the use of another crystalloid. This result is comparable to the NSS group at 31%, which has higher re-shock rates. In contrast, the NSS group had the tendency to use another crystalloid (AIES) in 12% of its patients because of NSS's higher chloride content, thus causing further increase in the serum chloride levels leading to the development of hyperchloremic metabolic acidosis. Because of higher re-shock rates in the NSS group, it needed more transfusion of packed red blood cells and use of inotropes. The need for mechanical ventilation was comparable in the NSS and LRS groups at 13.8% and 14%, respectively, as both groups have 50% of its patients having episodes of re-shocks.

Table 6. Summary on the Use of Colloid, Another Crystalloid, Packed Red Blood Cells Transfusion, Inotropes, and Mechanical Ventilation

	Acetated Isotonic Electrolyte Solution (n = 58)	Normal Saline Solution (n = 58)	Lactated Ringer's Solution (n = 50)	p value
Use of Colloid	11 (18.97%)	18 (31.03%)	17 (34%)	0.1719
Use of Another Crystalloid	0	7 (12.07%) – AIES	0	0.5078
pRBC Transfusion	4 (6.89%)	9 (15.52%)	6 (12%)	0.3416
Use of Inotropes	6 (10.34%)	13 (22.41%)	6 (12%)	0.1476
Use of Mechanical Ventilator	1 (1.72%)	8 (13.79%)	7 (14%)	0.0875
Need for Dialysis	0	1 (1.72%)	1 (2%)	0.9928

The length of stay in all the three IVF groups was almost the same and was not statistically significant.

The PELOD-2 score is based on the initial white blood cell count and platelet count, Glasgow Coma Scale, pupillary reaction, serum lactate, mean airway pressure, serum creatinine, PaO_2, $PaCO_2$, and the use of mechanical ventilation. But when a parameter is not measured, that parameter is considered normal. PELOD-2 score predicts the probability of death

– the higher the score, the higher the probability that the patient may expire. Among the three IVF groups, the NSS group had the highest PELOD-2 score, thus having the highest probability of mortality as shown in Table 7. This was consistent with the actual number and percent mortality in the NSS group with 3 mortalities (5.2%) as compared to the LRS group with 2 mortalities (4%) and the AIES group with none.

Table 8. PELOD-2 Scores and Number of Mortalities

	Acetated Isotonic Electrolyte Solution (n = 58)	**Normal Saline Solution (n = 58)**	**Lactated Ringer's Solution (n = 50)**	**p value**
PELOD-2 Scores Upon Admission				
1 month – 8 years old				
• Mean	3.88	4.57	4.05	0.06331
• Probability of Death	0.83	1.14	0.89	
9 – 18 years old				
• Mean	3.63	5.35	3.13	**0.031225**
• Probability of Death	0.73	1.63	0.58	
Mean PELOD-2 Scores	3.79	4.84	3.5	0.053307
Mortality				
• 1 month – 8 years old	0	2	1	0.2352
• 9 – 18 years old	0	1	1	
Total Mortality	0	3	2	
% Mortality	0	5.17%	4%	

Patients who were 1 month – 8 years old also showed the highest PELOD-2 scores, thus having the highest probability of mortality. Moreover, those patients who eventually expired have PELOD-2 scores of more than 10, except for 1 patient with a score of 5.

Multivariate linear regression was done to eliminate heterogeneity among variables, specifically that of the demographics of the patient. All the demographics were not statistically significant after regression analysis, hence considered homogenous.

Table 9. Summary of the clinical and laboratory parameters, and management of the mortalities among the NSS and LRS Groups

		Age, years	Gender	Actual Body Weight, kg	Ideal Body Weight, kg	Interpretation	Initial BP, mmHg	Initial HR, bpm	Initial pH level	Initial HCO_3 level	Initial ALT, mmol /L	Initial AST, mmol/L	Initial Na level, mmol/L	# of Re-shocks
NSS	**1**	4	F	28	16	Obese	90/60	160	7.34	10.9	548	629	128	3
	2	5	M	21	18	Normal	0	180	7.34	12.9	113	444	117	2
	3	11	F	43	36	Overweight	Palp 60	130	7.3	16.2	230	292	134	3
LRS	**1**	8	M	30.5	25.5	Overweight	Palp 90	140	7.12	16.6	220	237	145	2
	2	14	F	50	46.5	Normal	0	140	7.28	7.8	498	1364	143	5

		Initial Hct	Repeat Hct (% Change)	PRBC Transfusion	Initial WBC Count	Initial Platelet Count	Dengue Serology Test	Mechanical Ventilation	Initial Creatinine Level	Initial BUN Level	Dialysis	% Fluid Over load	GCS
NSS	**1**	50	40 (20%)	Yes	7.2	12	IgM +	Yes	Elevated	Normal	No	20	13
	2	49	31 (37%)	Yes	6	19	NS1Ag +	Yes	Normal	Normal	No	12.5	7
	3	53	36 (32%)	Yes	5.7	40	NS1Ag +	Yes	Normal	Low	No	43	12
LRS	**1**	45	37 (18%)	Yes	10.6	75	NS1Ag +	Yes	Elevated	Normal	Yes	12.6	12
	2	44	36 (18%)	Yes	8.2	10	IgM +	Yes	Normal	Normal	No	19	13

DISCUSSION

Crystalloid solutions remain to be the first line in the fluid resuscitation among dengue patients. But the choice of which intravenous fluid to be used in the initial resuscitation plays a very important role as it influences the course of dengue infection. With the introduction of another balanced crystalloid solution, there are limited local studies comparing its efficacy versus what is recommended based on the dengue fluid resuscitation protocol of the WHO and PPS. In this study, the use of acetated isotonic electrolyte solution produced better outcomes than normal saline and lactated Ringer's solutions.

In our country, there is no study conducted on the use of acetated isotonic electrolyte solution in dengue. To date, this is the first study conducted comparing its use versus the conventional use of normal saline and lactated Ringer's solution in dengue.

Theoretically, both acetated isotonic electrolyte solution and lactated Ringer's solution are both balanced crystalloid solutions and normal saline is not considered normal because of its high sodium content. The plasma-expanding capacity of these crystalloids is related to the sodium concentration.[8] However, all crystalloid fluids have no additional effect on the plasma volume since most are likely lost from the circulation because of sodium's molecular weight and vascular leakage in dengue. But of the three crystalloid intravenous fluids being studied, acetated isotonic electrolyte solution performed better as compared to both normal saline and lactated Ringer's solutions.

For the primary outcome, despite having more patients with hemoconcentration, narrower pulse pressure, and acidosis upon initial presentation, acetated isotonic electrolyte solution had the shortest time to achieve initial and sustained cardiovascular stability among severe dengue patients who did not have re-shock. It also needed less fluid volume to establish initial and sustained cardiovascular stability. In this study, these were attributed to the buffer system and electrolyte concentration, specifically that of sodium and chloride concentrations.

The buffer system of any crystalloid solution is important in the cellular level as it maintain the normal cellular physiology in varied conditions. It resists rapid changes in the blood pH. Acetated isotonic electrolyte solution has acetate and malate as the buffer system while lactated Ringer's solution has lactate. However, normal saline solution does not have any buffer systems, thus called unbuffered solution. Due to severe liver insufficiency in dengue patients, the metabolism of lactate in the liver produces more lactic acid, making the pH more acidotic, hence, worsening the pH level in patients with circulatory insufficiency. The advantage of acetate and malate of isotonic electrolyte solution is that they are not primarily metabolized in the damaged liver in dengue patients and they are converted to bicarbonate ions. This study showed that a crystalloid solution containing acetate, in addition with malate, was more effective in restoring blood pH and plasma bicarbonate within the normal range among patients with severe dengue and metabolic acidosis. This is beneficial among dengue patients because acidosis produces various deleterious effects in the different organ systems like the renal and respiratory systems, thus increasing risk of developing complications which may be prevented by using the most appropriate intravenous fluid on the initial recognition of shock.

Another advantage of the acetated isotonic electrolyte solution is its electrolyte concentration. Based on previous studies for fluid resuscitation, sodium concentration of the intravenous fluid must be at 130 – 155mmol/L. However, plasma sodium concentration is just at 135 – 145mmol/L. An ideal fluid for resuscitation must have electrolyte contents that are close enough to that of plasma. Hence, what is considered as normal saline is not actually normal and is sometimes termed as unbalanced crystalloid.The term balanced crystalloid solution was introduced as it closely resembles the physiologic level of serum sodium concentration. Both acetated isotonic electrolyte solution and lactated Ringer's solution are

balanced crystalloid. However, lactated Ringer's solution has sodium concentration of 130mmol/L as compared to acetated isotonic electrolyte solution which has 145mmol/L sodium concentration. With this, acetated isotonic electrolyte solution is the betterfluid of choice.

Aside from sodium, chloride concentration is also important. The chloride concentration of lactated Ringer's solution is within the physiologic level. However, the buffer system seemed to be the problem in dengue patients with hepatic damage. Between the three crystalloids, normal saline has the highest chloride concentration. The chloride load of an intravenous fluid contributes to the development of hyperchloremic metabolic acidosis. In this study, this was evident on the normal saline group with increased levels of chloride and metabolic acidosis. The presence of hyperchloremic acidosis can lead to acute kidney injury and at times, needing renal replacement therapy. In this study, this was comparable between the normal saline solution and lactated Ringer's solution groups in which as much as 2% of the patients required dialysis.

For the secondary outcomes, acetated isotonic electrolyte solution had less tendencies to have re-shock with minimal use of colloids and inotropes, and blood transfusion during re-shock, and less probability of requiring mechanical ventilator. In this study, common risk factors for re-shock were patients who presented with hypotension or profound shock with pulse pressure of less than 20mmHg and hemoconcentration on initial presentation. This was the same finding in the previous study by Ngo et al in 2001 in which patients with profound shock with pulse pressure of less than 10mmHg developed subsequent and prolonged shocks.[8]More re-shocks were noted in the normal saline group hence, more use of inotropes, blood transfusion, use of a balanced crystalloid, which is acetated isotonic electrolyte solution, and more probability that the patient get intubated and required mechanical ventilation, which was comparable to that of the lactated Ringer's solution group. In this study, this was attributed to the presence of hyperchloremic acidosis hence more adverse outcomes of acidosis and hyperchloremia. However, in this study, the normal saline group has the shortest time to achieve initial and sustained cardiovascular stability in patients who have re-shock. This was closely followed by the acetated isotonic electrolyte solution and then the lactated Ringer's solution. The use of colloid was highest in the lactated Ringer's solution group and this finding was again consistent with the study done by Ngo et al.[8]

Mortality in dengue cases is multifactorial. However, in this study, patients who expired from the normal saline solution and lactated Ringer's solution groups have the following common risk factors as summarized in Table 18 – overweight to obese, profound shock with associated tachycardia, presence of metabolic acidosis, elevated transaminase levels, multiple re-shocks requiring inotropic support and the use of mechanical ventilation, active plasma leakage with associated hemoconcentration, more than 10% decrease from the initial hematocrit level and needed packed red blood cell transfusion, Glasgow coma scale score of 13 and less, and fluid overload of more than 10%. This finding was consistent with the study done by Almas et al in 2010 in which the predictors of mortality among dengue patients included bleeding, altered consciousness and profound shock on initial presentation.[26]

Furthermore, cost-effectiveness of these crystalloids should be put into consideration since most of the patients were charity patients. In this institution, a liter of acetated isotonic electrolyte solution costs PhP250 as compared to Php60 per liter of normal saline and lactated Ringer's solutions. However, because more adverse effects were determined in using normal saline and lactated Ringer's solutions in severe dengue despite having a lower price, using acetated isotonic electrolyte solution iscost-effective as it has more favorable outcome.

CONCLUSION AND RECOMMENDATION

In conclusion, acetated isotonic electrolyte solution is more effective as compared to normal saline solution and lactated Ringer's solution in the initial fluid resuscitation among patients with severe dengue. It must be used as the fluid of choice in severe dengue patients who need fluid resuscitation. Its use produces less re-shock, less use of cardiovascular support, less rescue colloid use, more stable acid-base balance, less bleeding and blood transfusion, and less need for mechanical ventilation and dialysis. We recommend that a randomized control trialcomparing these crystalloids with larger number of patients with severe denguemust be conducted.

BIBLIOGRAPHY/REFERENCES

1. Bravo L, Roque VG, Brett J, Dizon R, L'Azou M. Epidemiology of dengue disease in the Philippines (2000–2011): a systematic literature review. PLoSNegl Trop Dis. 2014;8(11):e3027.

2. Annual dengue data in the western pacific region, WHO Western Pacific Regional Office 2010.

3. Arima Y, Matsui T. Epidemiologic update on the dengue situation in the Western Pacific Region 2010. WPSAR. 2011;2(2):4-8.

4. Philippine Pediatric Society. Revised dengue guidelines. October 2012.

5. Santi M, Lava SA, Camozzi P, Giannini O, Milani GP, Simonetti GD, et al. The great fluid debate: saline or so-called "balanced" salt solutions. Ital J Pediatr. 2015;41:47.

6. Bartels K, Thiele RH, Gan TJ. Rational fluid management in today's ICU practice. Crit Care. 2013;17 Suppl1:S6.

7. Varrier M, Ostermann M. Fluid composition and clinical effects. Crit Care Clin. 2015;31:823–37.

8. Ngo NT, Cao XT, Kneen R, Wills B, Nguyen VM, Nguyen TQ, et al. Acute management of dengue shock syndrome: a randomized double blind comparison of 4 intravenous fluid regimes in the first hour. Clin Infect Dis. 2001;32(2):204–13.

9. Cifra HL, Velasco JNJ. A comparative study of the efficacy of 6% Haes-Steril and Ringer's lactate in the management of dengue shock syndrome. Crit Care Shock. 2003;6:95-100.

10. Wills BA, Nguyen MD, Ha TL, Dong TH, Tran TN, Le TT, et al. Comparison of three fluid solutions for resuscitation in dengue shock syndrome. N Eng J Med. 2005;353:877–89.

11. Karyanti MR, Satari HI, Sjarif DR. The effect of Ringer's acetate versus Ringer's lactate on aminotransferase changes in dengue hemorrhagic fever. PaediatrIndones. 2005;45:81-6.

12. Jalac SLR, de Vera M, Alejandria MM. The use of colloids and crystalloids in pediatric dengue shock syndrome: a systematic review and meta-analysis. PJMID. 2010;39:14-27.

13. Akech S, Ledermann H, Maitland K. Choice of fluids for resuscitation in children with severe infection and shock: systematic review. BMJ. 2010;341:c4416.

14. Hofmann-Kiefer KF, Chappell D, Kammerer T, Jacob M, Paptistella M, Conzen P, et al. Influence of an acetate- and a lactate-based balanced infusion solution on acid base physiology and hemodynamics: an observational pilot study. Eur J Med Res. 2012;17:21.

15. Soegijanto S, Sari D, Yamanaka A, Kotaki T, Kameoka M, Konishi E. Awareness of using Ringer lactate solution in dengue virus infection could induce severity. IJTID. 2013;4(4):35-41.

16. Perel P. Colloids versus crystalloids for fluid resuscitation in Dengue fever patients – a review. For WHO Secretariat. 2013.

17. Somasetia DH, Setiati TE, Sjahrodji AM, Idjradinata PS, Setiabudi D, Roth H, et al. Early resuscitation of dengue shock syndrome in children with hyperosmolar sodium-lactate: a randomized single-blind clinical trial of efficacy and safety. Crit Care. 2014;18:466.

18. Córtes DO, Rayo Bonor A, Vincent JL. Isotonic crystalloid solutions; a structured review of the literature. Br J Anesth. 2014;112(6):968–81.

19. McDermid RC, Raghunathan K, Romanovsky A, Shaw AD, Bagshaw SM. Controversies in fluid therapy: type, dose and toxicity. World J Crit Care Med 2014;3(1):24-33.

20. Lam PK, Hoai Tam DT, Dung NM, Hanh Tien NT, Thanh Kieu NT, Simmons C, et al. A prognostic model for development of profound shock among children presenting with dengue shock syndrome. PLoS ONE. 2015;10(5):e0126134.

21. Raghunathan K, Bonavia A, Nathanson BH, Beadles CA, Shaw AD, Brookhart MA, et al. Association between initial fluid choice and subsequent in-hospital mortality during the resuscitation of adults with septic shock. Anesthesiology. 2015;123:1385-93.

22. Varrier M, Ostermann M. Fluid composition and clinical effects. Crit Care Clin. 2015;31:823-37.

23. Chameides L, Samson R, Schexnayder S, Hazinski MF. Pediatric Advanced Life Support Provider Manual. American Heart Association; 2011.

24. Handbook for clinical management of dengue. WHO 2012.

25. Hung N. Fluid management for dengue in children. PediatrInt Child Health. 2012;32(S1).

26. Almas A, Parkash O, Akhter J. Clinical factors associated with mortality in dengue infection at a tertiary care center. Southeast Asian J Trop Med Public Health. 2010;4(2):333-340.

ELECTROCARDIOGRAM AS A PREDICTIVE TOOL FOR THE SEVERITY AND CLINICAL COURSE OF PEDIATRIC DENGUE INFECTIONS

RIGIL MARIQUIETA FE P. SIAZON, MD
LEAH PATRICIA M. ARCEO-PLUCENA, MD

ABSTRACT

BACKGROUND: Dengue is a mosquito-borne viral disease that has been a global burden especially in the tropical regions. Cardiac involvement has been discussed in several studies. This is a pilot study to identify electrocardiogram abnormalities and correlate these with the severity of dengue illness.

OBJECTIVES: To evaluate the efficiency of electrocardiogram as a predictive tool for the severity and clinical course of pediatric dengue infections.

METHODS: This prospective cohort study was conducted at the Philippine Children's Medical Center from August to October 2017. A total of 325 dengue consults were seen at the pediatric emergency room during the study period. Of these, 66 pediatric patients fulfilled the inclusion criteria for this study. Serial pediatric electrocardiograms (ECG) were performed on days 1, 7, and 14 afebrile. The ECGs were interpreted according to rhythm, axis and duration of waveforms and intervals. These ECG interpretations were subsequently correlated to the patients' dengue severity and clinical course.

RESULTS: Rhythm abnormalities were found in 15 out of the 66 dengue patients for an overall incidence of 23%. All the rhythms were benign and self-limiting, including sinus bradycardia, tachycardia, and first-degree atrio-ventricular block. Majority of the dengue patients had sinus rhythm (42 of 66, 64%), and of these, 8 had heart rates at the lower limits of normal range for age (12%). 23 percent of abnormal rhythms were detected on day 1 of illness. All rhythm abnormalities resolved by day 14 of afebrile.

CONCLUSION: The series of ECG did not show clinically significant or life-threatening arrhythmias during the patients' dengue illness in this cohort. All arrhythmias were benign and self-limiting, regardless of dengue severity. The ECG did not correlate well with and cannot be utilized to predict the clinical course of the dengue illness.

RECOMMENDATIONS: A retrospective comparative study is recommended to determine predictability of dengue severity using ECG. A bigger cohort of dengue patients may detect significant arrhythmias not caught in this study.

KEYWORDS: Electrocardiogram, ECG, dengue with warning signs, severe dengue, bradycardia

INTRODUCTION

Dengue, a mosquito-borne viral disease, has been a global burden especially in the tropical regions. In the Philippines, it is already a year-round endemic disease with high morbidity and mortality among pediatric patients. It has been extensively studied with frequent updates on diagnosis, new case classification, and management. However, parameters and diagnostic exams to predict which dengue patients will have complications and more severe disease have not been extensively elucidated. Routine laboratory examinations are requested such as complete blood count, NS1Ag, and dengue blot (IgM/ IgG) to confirm dengue infection. However, there is no study that confirms the exact

correlation of dengue severity and a particular examination.

Cardiac complications arising from dengue infections are very rare. There are only a few studies, with its incidence ranging from as low as 6.7%, up to 36%.[1]These studies also describe dengue shock as being due to cardiovascular impairment and permeability caused by the viremia.

Dengue myocarditis is a relatively uncommon occurrence, with one study reporting it at 13.9%.[2]Moreover, this cardiac sequela has been found to be self-limiting, and in some cases subclinical, proven only by elevated cardiac enzymes that eventually resolve with the illness.

More commonly, dengue infections have been found to be associated with cardiac arrhythmias at various times during illness, most of which are benign. The underlying mechanisms for these electrical abnormalities can be due to altered autonomic tone, electrolyte and calcium derangements, or subclinical myocarditis. However, these electrical abnormalities in dengue have not been adequately studied.[3]The electrocardiogram (ECG) is a readily available diagnostic tool in most tertiary hospitals. In this study, the electrocardiographic findings were documented on different days in the course of the dengue illness to determine ifECG can be used to predict the clinical course outcome and severity of dengue.

According to the World Health Organization (WHO) Global Strategy for Dengue Prevention and Control, dengue ranks as the most important mosquito-borne viral disease in the world in 2012, with a 30-fold increase in incidence over the past 5 decades. [4] The literature review done by Bravo, et.al, showed that there is a consistent rise in the incidence of dengue disease from 2001-2011.[5] A sharp increase was noticeable in the year 2010 as provided by both the Department of Health and the World Health Organization.Dengue disease was recorded highest among pediatric patients aged 5-14 years old with dengue-related deaths highest among children <9 years old.

In 2009, the WHO classified dengue cases as dengue with or without warning signs and severe dengue parallel to the 1997 classification of dengue fever, dengue hemorrhagic fever and dengue shock syndrome.[6] Classifying dengue without warning signs requires febrile episodes accompanied by any two of non-specific symptoms such as nausea, vomiting, rash, aches, pain, leukopenia, or a positive tourniquet test. Warning signs include abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation (i.e. ascites, pleural effusion), mucosal bleed, lethargy, restlessness, liver enlargement >2 cm, and a laboratory test showing increase in hematocrit concurrent with a rapid decrease in platelet count. Severe dengue is defined as having either severe plasma leakage leading to shock and/or fluid accumulation with respiratory distress, orsevere bleeding as evaluated by a clinician, or severe organ involvement (i.e. elevated transaminases, impaired consciousness, heart failure or failure of other organs).

Cardiac involvement in dengue include function almyocardial impairment, arrhythmias, and myocarditis that contribute tothe overall severity of the hemodynamic compromise. Yacoub, et.al proposed viral and immune mechanisms involved in dengue compromising the cardiac and vascular system. Dengue virus (DENV) is taken up into macrophages resulting to T-cell activation and release of vasoactive and pro-inflammatory cytokines affecting capillary leak and possibly myocardial impairment. The interaction of NS1 and the glycocalyx layer of the vascular endothelium is thought to increase capillary permeability. The resulting plasma leakage can contribute to the cardiac dysfunction in the form of reduced preload, altered coronary microcirculation, and myocardial interstitial edema. Altered intracellular calcium homeostasis has also been demonstrated in dengue infected myotubes.[7] Dengue myocarditis is diagnosed by elevated cardiac biomarkers with supplemental echocardiography of subclinical findings such as arrhythmias, and symptoms of dyspnea and chest pain.[8] However, endomyocardial biopsy is still the gold standard in the diagnosis of myocarditis.

Yacoub, et. al., explained the reason behind cardiac dysfunction in dengue. The mechanism is shared by other studies done where high circulating proinflammatory cytokines causemyocardial depression as well as capillary permeability. Other potential mechanisms include altered intracellular calcium homeostasis and coronary hypoperfusion.[9] Salgado, et.al. explained that the increased resting or diastolic calcium level in muscle cells during dengue infectionmay be responsible for the arrhythmias and altered contractile function of the myocardium.[2]

In a case control study done by Lateef, et.al, 3 out of 50 cases presented with bradycardia. Echocardiogram (2D-echo) was done and only 1 showed mitral valve prolapse with mild regurgitation.[10]

One study in Sri Lanka by Wichmann, et.al. showed that 25% of dengue patients presented with one or more elevated markers of myocardial injury, specifically myoglobin, creatine kinase MB (CK-MB), and troponin T.[11]

In a literature review by Wiwanitkit, out of the thousand cases of dengue hemorrhagic fever(DHF) in Thailand, only 2 were reported as dengue myocarditis. Both were pediatric patients and presented with bradycardia and hypotension a day after recovering from DHF. ECG showed junctional rhythm.[12]A case report by Shah was an 11-year old boy who was tachycardic (110 beats/minute), hypotensive (85/50 mmHg), with prolonged capillary refill and bilateral basal crepitations. He was managed as a case of dengue. CK-MB was elevated, and 2d-echo showed left ventricular dilatation and systolic dysfunction with a fractional shortening of 22%. After 3 months, he was asymptomatic, and a repeat 2d-echo showed improved fractional shortening of 37%.[13]

In a cohort study by Satarasinghe, et.al, out of the 217 patients with dengue fever, 85% underwent echocardiography. Evidence of myocarditis was seen in 24% with an age range of 12-65 years old. All had relative bradycardia of 50-60 beats/minute. [14]

Gupta, et.al claimed a subclinical cardiac involvement in DHF. In 28 patients, 14% had bradycardia and 14% had 2d-echo finding of grade 1 diastolic dysfunction. In other cases, 2d-echo was normal but cardiac enzymes of CK-MB and troponin T were significantly increased, inferring a subclinical cardiac involvement.[15]

According to Yacoub, et.al, rhythm disturbances such as bradycardia were thought to occur primarily in the recovery phase. Heart rate is relatively lower at peak temperature in dengue patients compared to other illnesses with febrile episodes. ECG findings were reported to be transient and non-specific such as sinus bradycardia, AV block, T-wave, and ST segment abnormalities. In a 24-hour Holter monitoring study of 35 children in the recovery phase of dengue, 29% had ECG abnormalities, 27 with bradyarrhythmias (first and second degree heart block), atrial/ventricular ectopic beats, and tachyarrhythmias, including atrial fibrillation. [7]

Thecase series of Yantie, et.al, reported 10 patients with DHF who had ECG abnormalities, 3 patients showed sinus arrhythmias, 3 patients showed sinus bradycardia, 1 patient showed sinus tachycardia without fever, 2 patients showed a first-degree AV block and 1 patient showed a second-degree AV block Mobitz type I. Their abnormal ECG findings were mostly noted at days 6-7 of illness. Normal ECG was then documented at days 10-14 of illness.[16]Wali, et.al, did a prospective studyin Thailand. It reported that of 35 children, at least 24 hours after defervescence, 10 patients (29%) had abnormalities of rhythm, first-degree AV block, Mobitz type I second-degree AV block, atrial ectopics, and ventricular ectopics.[17]

La-Orkhun, et. al., used Holter monitoring to assess cardiac rhythm and rate among children (11.7 ± 2.3 years) with dengue infection. During the convalescent phase, 29% of patients had ECG abnormalities including sinus arrhythmias, first-degree and Mobitz type I second-degree AV block, and atrial and ventricular ectopic beats.[18]

Hussein, et. al., made a cross-sectional study among 17 diagnosed pediatric dengue patients who underwent electrocardiogram. Findings of P wave amplitude was getting shorter as the severity of dengue infection increased. While P wave duration increased slightly with increased severity of dengue infection. There was a significant increase in PR interval as the severity of dengue infection increased. The same was also true for ST and QTc wave.[19]There are changes noted as severity of disease increased, however all values are still in the normal range.

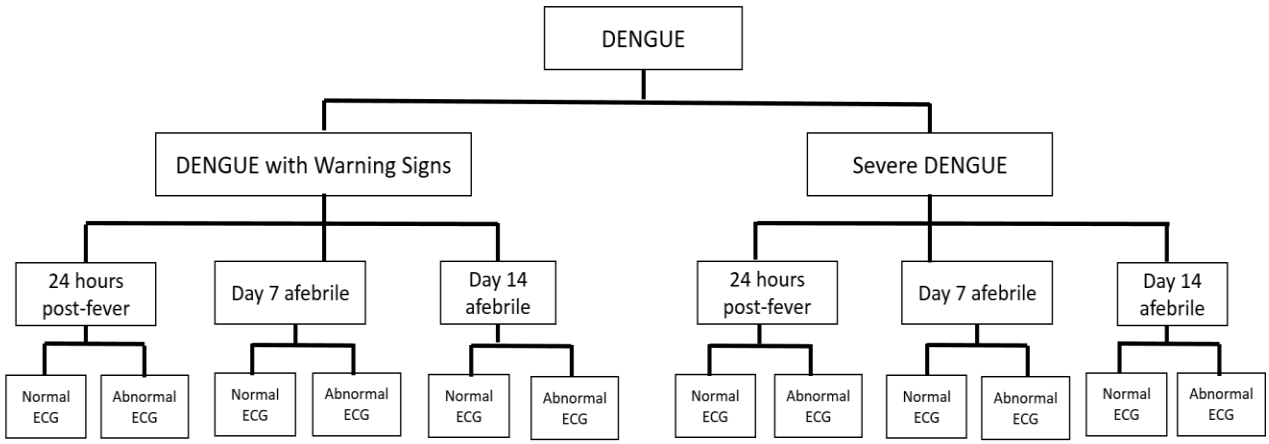
The cited journals provide data of dengue affecting the cardiovascular system. Moreover, there are abnormal ECG findings recorded specifically occurring when fever resolves. Whatever abnormal finding is recorded, this spontaneously resolves with null effect on the clinical course of the patient. Still, these ECG findings can also help predict the outcome of dengue.

METHODOLOGY

This was a prospective cohort study; included were children 5-18 years old; emergency room patients with a presumptive diagnosis of dengue; and confirmed diagnosis either with positive Dengue NS1Ag and/or Dengue IgM/ IgG. Excluded were patients with pre-existing congenital or acquired heart disease; patients with pre-existing cardiac arrhythmias of hemodynamic significance; patients with known hematologic and oncologic disorders; patients with co-existing infection aside from dengue; patients with congenital disorders and syndromes; and patients diagnosed with dengue but admitted day two or more of fever lysis.

Subjects were 66 consecutively admitted patients at the Philippine Children's Medical Center (PCMC) from August to October 2017. They were classified as either with Warning Signs or Severe Dengue.

Description of the Study Procedure:



This prospective in-patient cohort study was conducted at the Philippine Children's Medical Center for a period of three months from August to October 2017. All patients admitted at the Emergency Room, a total of 325, with a provisional diagnosis of dengue infection were screened for possible inclusion in the study and only those with serologic confirmation (NS1 antigen, Dengue blot) were included.

The patients were properly classified according to WHO classification of dengue severity: without warning signs (DWOW), with warning signs (DWW), and severe dengue (DS).The baseline vital signs on admission were recorded at the ER, particularly temperature. Existing dengue management protocols were implemented, including laboratory tests and

fluid resuscitation, and left upon the discretion of ER team.

After securing consent, ECG were performed for each patient on the first day, seventh day and fourteenth day after fever lysis (D1, D7 and D14, respectively). If patients were discharged prior to day 7 afebrile, follow-up was scheduled on the date coinciding with the repeat ECG. The same is true for day 14 afebrile.

Each pediatric 15-Lead ECG was performed at the Cardiac Diagnostic Center, by either one of the ECG technicians using the Mortara ELI 250c ECG machine. The ECG was recorded and printed on standard ECG paper. The ECG was performed and recorded while the patient was asleep, or awake but calm and quiet. Crying and agitated patients had the procedure deferred until optimal conditions were achieved and was performed within the 24-hour period of the designated day. The ECG was read and interpreted by a pediatric cardiologist. These ECG readings were tabulated and analyzed for statistical significance according to dengue severity and day of illness. All ECGs were collated according to day of afebrile interpretation of rhythm, axis and duration of waveforms, and intervals were tabulated according to dengue severity.

The data gathered were recorded and processed prior to analysis using the MS Excel 2010. Control numbers were assigned to each item. Binary coding was employed for variables of normal and abnormal results. The categorical variables were coded using numerical numbers. The scores were recorded in percentage. All variables were presented in frequency and percentage cross tabulations. The association of each variable to the type of dengue severity was assessed using Pearson Chi-Square Test for Independence or Adjusted Fisher's Exact Test. Significance level was set to 5%. Comparisons of means of ECG values between types of dengue severity were evaluated using Mann-Whitney test. Proportion, mean differences and associations were deemed significant if corresponding p-values of statistics did not exceed 0.05. SPSS version 20.0 was used to output all necessary results.

RESULTS

A total of 325 dengue consults were seen at the pediatric emergency room during the study period. Out of the 68 patients, 63 were diagnosed to have severe dengue (SD) while only 5 cases were registered to have dengue with warning signs (DWS). There were 2 cases who dropped out as early as day 1 afebrile due to lack of consent. Mean age for both groups was 10 years old, proving no significant difference in the mean and proportion of age distribution in between groups ($p=0.372 > 0.05$). More female patients were found in both DWS and DS groups. Despite disparity, no sufficient evidence was proven linking gender and severity of dengue ($p=0.413 > 0.05$). (Table 1)

Table 1. Demographic Profile of the Sample

Demographic Profile		Type of Dengue Severity						P-Value
		Severe Dengue		Dengue with Warning Signs		Total		
		Frequency	Percent	Frequency	Percent	Frequency	Percent	
Age Group	5-8	1	20%	22	35%	23	34%	0.372
	9-12	2	40%	21	33%	23	34%	
	13-15	2	40%	9	14%	11	16%	
	16-18	0	0%	11	18%	11	16%	
	Total	5	100%	63	100%	68	100%	
Age (Mean ± SD)		*10.4 ± 3.21*		*10.6 ±3.94*				

Table 1. (Continuation)

Demographic Profile		Type of Dengue Severity						P-Value
		Severe Dengue		Dengue with Warning Signs		Total		
		Frequency	Percent	Frequency	Percent	Frequency	Percent	
Sex	Male	2	40%	36	57%	38	56%	
	Female	3	60%	27	43%	30	44%	0.648
	Total	5	100%	63	100%	68	100%	
IgM	Yes	4	80%	39	62%	43	63%	
	No	1	20%	24	38%	25	37%	0.645
	Total	5	100%	63	100%	68	100%	
IgM/ IgG	Yes	4	80%	30	48%	34	50%	
	No	1	20%	33	52%	34	50%	0.356
	Total	5	100%	63	100%	68	100%	
Ns1Ag	Yes	1	20%	23	37%	24	35%	
	No	4	80%	40	64%	44	65%	0.649
	Total	5	100%	63	100%	68	100%	
IgG	Yes	4	80%	31	49%	35	52%	
	No	1	20%	32	51%	33	49%	0.357
	Total	5	100%	63	100%	68	100%	

Table 2 reveals that a normal ECG finding does not necessarily predict lesser dengue type among patients; nor and abnormal ECG finding to severity of dengue.

Table 2. Association of General ECG Finding and Level of Dengue Severity at Day 1, 7, and 14 Afebrile

Period	ECG Finding	Severe Dengue		Dengue with Warning Signs		Total		P-Value
		Frequency	Percent	Frequency	Percent	Frequency	Percent	
Day 1	Normal	5	100%	46	75%	51	77%	
	Abnormal	0	0%	15	25%	15	23%	0.15
	Total	5	100%	61	100%	66	100%	
Day 7	Normal	1	50%	14	88%	15	83%	
	Abnormal	1	50%	2	12%	3	17%	1
	Total	2	100%	16	100%	18	100%	
Day 14	Normal	0	0%	8	89%	8	89%	
	Abnormal	0	0%	1	11%	1	11%	NA
	Total	0	0%	9	100%	9	100%	

Table 3 shows that all 5 cases of severe dengue and 46 diagnosed with dengue with warning signs had sinus rhythm and sinus arrhythmia (77%)on Day 1. Among the 61

dengue with warning signs patients, the most prevalent abnormal ECG finding sinus bradycardia (n=6, 9%), generalized flattened T waves (n=3, 5%), and bradyarrhythmia (n=2, 3%). However, differences in proportions were not proven to be statistically significant due to absence of cases in one group. Similarly, no sufficient evidence was proven to determine significant differences in the mean ECG values in between groups. (Table 3.1)

Table 3. Association of Findings from the Two Types of ECG and Type of Dengue Severity at Day 1 Afebrile

ECG Finding	Type of ECG Finding/Reading at Day 1	Type of Dengue Severity				Total	P-Value
		Severe Dengue		Dengue with Warning Signs			
		Frequency	Percent	Frequency	Percent		
Normal	Generalized flattened T waves	0	0%	0	0%	0	NA
	Prolonged QTc interval	0	0%	0	0%	0	
	Relative right axis deviation	0	0%	0	0%	0	
	Sinus arrhythmia	0	0%	9	20%	9	
	Sinus bradycardia	0	0%	0	0%	0	
	Sinus rhythm	5	100%	37	80%	42	
	Sinus tachycardia	0	0%	0	0%	0	
	Total	**5**	**100%**	**46**	**100%**	**51**	
Abnormal	Generalized flattened T waves	0	0%	3	20%	3	NA
	Prolonged QTc interval	0	0%	1	7%	1	
	Relative right axis deviation	0	0%	1	7%	1	
	Sinus arrhythmia	0	0%	0	0%	0	
	Sinus bradyarrhythmia	0	0%	2	13%	2	
	Sinus bradycardia	0	0%	6	40%	6	
	Sinus rhythm	0	0%	0	0%	0	
	Sinus tachycardia	0	0%	2	13%	2	
	Total	**0**	**0%**	**15**	**100%**	**15**	

Table 3.1 Comparisons of Means between Severe and Dengue with Warning Signs at Day 1 Afebrile

	Type of Dengue Severity	N	Mean	Std. Deviation	P-Value
PRDay1	Severe Dengue	5	0.14	0.02	0.226
	Dengue with Warning signs	61	0.15	0.02	
RateDay1	Severe Dengue	5	79.40	14.76	0.538
	Dengue with Warning signs	60	74.77	13.96	
QRSDurationday1	Severe Dengue	5	0.08	0.03	0.887
	Dengue with Warning signs	61	0.08	0.01	
QTADurationDay1	Severe Dengue	5	0.31	0.15	0.436
	Dengue with Warning signs	61	0.37	0.03	
QTCDay1	Severe Dengue	5	0.42	0.02	0.085
	Dengue with Warning signs	61	0.40	0.02	

Note: PR interval = seconds, mean±SD = Mean±Standard Deviation; QRS duration = seconds, mean ± standard deviation; rate = bests per minutes

Table 4 shows that on Day 7 afebrile, there were two patients with severe dengue was 2, one having a normal sinus rhythm and the other with first degree AV block. On the other hand, cases of patients with dengue with warning signs were reduced from 61 to 16. Majority (n=10, 71%) had normal ECG findings. No p-value was calculated due to absence of cases in one group. Similarly, no sufficient evidence was gathered to determine significant differences in the mean ECG values between groups. (Table 4.1)

Table 4. Association of Findings from the Two Types of ECG and Type of Dengue Severity at Day 7 Afebrile

ECG Finding	Type of ECG Finding/Reading at Day 7	Type of Dengue Severity				Total	P-Value
		Severe Dengue		Dengue with Warning Signs			
		Frequency	Percent	Frequency	Percent		
Normal	Sinus rhythm	1	100%	10	71%	10	NA
	Sinus arrhythmia	0	0	4	29%	4	
	Total	1	100%	14	100%	14	
Abnormal	Prolonged QTc interval	0	0%	1	50%	1	NA
	Sinus arrhythmia	0	0%	0	0	0	
	Sinus tachycardia	0	0%	1	50%	1	
	First degree AV block	1	100%	0	0%	0	
	Total	1	100%	2	100%	2	

Table 4.1 Comparisons of Means between Severe and Dengue with Warning Signs at Day 7 Afebrile

	Type of Dengue Severity	N	Mean	Std. Deviation	P-Value
PRDay7	Severe Dengue	2	0.17	0.06	0.72
	Dengue with Warning signs	16	0.14	0.02	
RateDay7	Severe Dengue	2	86.50	10.61	0.44
	Dengue with Warning signs	16	80.94	8.35	
QRSDay7	Severe Dengue	2	0.07	0.01	0.60
	Dengue with Warning signs	16	0.07	0.01	
QTaDay7	Severe Dengue	2	0.35	0.04	0.29
	Dengue with Warning signs	16	0.37	0.03	
QTCDay7	Severe Dengue	2	0.41	0.01	1.00
	Dengue with Warning signs	16	0.41	0.03	

Note: PR interval = seconds, mean±SD = Mean±Standard Deviation; QRS duration = seconds, mean ± standard deviation; rate = beats per minute

On Day 14 afebrile, no more cases of severe dengue patients were recorded and only 9 patients were still diagnosed to have dengue with warning signs. Only 1 out of the 9 remaining had an abnormal ECG finding (incomplete right bundle branch block) while all the 89% (or 8 patients) had normal sinus rhythm (Table 5).

Table 5. Type of Dengue Severity per ECG Findings at Day 14 Afebrile

	Type of Dengue Dengue with Warning signs	
	Frequency	**Percent**
Sinus rhythm (Normal)	8	89%
Incomplete right bundle branch block (Abnormal)	1	11%
Total	9	100%

DISCUSSION

This study shows that there are ECG findings that deviate from normal in patients with dengue. Despite the absence of abnormal clinical findings on cardiac examination such as rate, rhythm or murmurs, subclinical ECG findings were noted. There were also varying duration of ECG intervals in relation to the day of afebrilethat can help predict cardiac involvement. The results do not point to an age predilection of dengue severity among pediatric patients. In terms of gender, there was no predilection as to the severity despite the predominance of female subjects with dengue with warning signs and severe dengue.

The findings in this study are consistent with the findings by Yacoub, et.al., with rhythm disturbances occurring primarily during the recovery phase and were observed to be transient and non-specific. This study similarly showed heart rates of low-normal levels during the recovery phase. This is also consistent with the study done in Thailand by Wali, et. al. where 24-hour post-defervescence findings were rhythm abnormalities, ectopies and AV blocks. This is also comparable to the study made by Yantie, et. al., where abnormal ECG findings were noted during days 6-7 illness, however the day of fever lysis was not indicated. The pathophysiology of the abnormal rhythm, as well as rate, is explained by proinflammatory cytokines causing myocardial depression, capillary permeability, and increase in resting calcium level.The mean heart rate in DWS was 74.77 ± 13.96 bpm in this study, comprising 12% of DWS cases with bradycardia during the recovery phase.

During day 7 afebrile, a decrease in the number of abnormal ECG findings was noted. The patients failed to follow-up for a repeat ECG. The reasons for failure to comply can be due to low follow-up rate in the outpatient or low positive reinforcement to encourage follow-up. First degree AV block in severe dengue, and prolonged QTc interval and sinus arrhythmia in dengue with warning signs were significant findings. The case with first degree AV block had severe dengue with a prior ECG of sinus rhythm. The other case of first degree AV block was a patient with dengue with warning signs who had an initial finding of sinus bradycardia. All these rhythms were interpreted as normal pediatric variants. This is in consistent with the findings of Yantie, et. al., where normal ECG findings seen during days 10-14 of illness.

During day 14 afebrile, there was a decrease in the number of patients who followed-up for the third and last ECG. All the remaining cases were dengue with warning signs. Among the 9 cases, one had an incomplete right bundle branch block. The previous reading of the said case during the days 1 and 7 afebrile was sinus rhythm. The 2 cases with first degree AV block during the day 7 afebrile failed to do the repeat ECG on day 14 afebrile, hence whether such findings resolved was not established.

The rhythm disturbances during day 1 afebrile were found to have spontaneously resolved as the days progressed. This is likeYantie, et. al. where normal ECG findings were recorded as the day of illness progressed and patients were already afebrile.

The difference inPR interval between the DWS and DS groups was not statistically significant. The PR interval mean (range) for

DWS was0.15 ± 0.02 (0.13 – 0.17) second and compared to0.14 ± 0.02 (0.12 – 0.16) second for the DS patients. Despite within normal levels, there are certain changes in ECG values notably more in DWS that might explain cardiac involvement. The difference in QRS duration was likewise not statistically significant between the two groups, in DS was 0.08±0.03 (0.0.05 to 0.1) second and for DWS was 0.08±0.01 (0.07 – 0.09) second. The recorded QTc interval for all cases were all within normal values. During days 7 and 14 afebrile, all the recorded heart rates were normal. The QRS duration was also normal.

CONCLUSION AND RECOMMENDATIONS

ECG can be used to assess the cardiac function of dengue infection. Rhythm findings included bradycardia, bradyarrhythmia, and sinus tachycardia which were observed consistently during the first day of recovery phase. Most of these findings were benign and self-limiting. The ECG values (i.e. PR and QRS duration), despite within normal levels provide changes in the verified values that might predict cardiac involvement and severity in dengue infection. As the days of afebrile progressed, the heart rate and PR-QRS intervals improved to normal values. The ECG did not correlate well with and cannot be utilized to predict the clinical course of the dengue illness.

This study only observed the type of ECG finding as a predictor to dengue severity. Failure to correlate the above variables was affected by a very limited sample size causing no patterns yet on the records. Whether ECG can be used to predict dengue severity needs further study to link the said variables.

Further research on the predictability of ECG to dengue severity is urged. A retrospective comparative study among dengue patients who underwent ECG and an active-control (standard diagnosis) is recommended first to determine predictability of the level of dengue severity using ECG as a tool to set a grounding evidence for succeeding protocols with higher statistical power and accuracy. After preliminary evidence is established, a prospective study may be utilized using randomized parallel study design, i.e., comparing two groups with the primary aim of investigating whether ECG could be used as a possible alternate diagnostic tool from standard predictor of dengue severity. ROC Analysis may be utilized to determine accuracy rate of the alternative method. Sample size may be computed by referring to the results of the retrospective study.

BIBLIOGRAPHY

1. Khongphatthanayothin A, Lertsapchareon P, Supachokchaiwattana P, La-orkhun V, Khumtonvong A, et al. Myocardial depression in dengue hemorrhagic fever: prevalence and clinical description. Pediatr. Crit. Care Med 2007; 8, 524–529.

2. Salgado DM, Eltit JM, Mansfield K, Panqueba C, Castro D, Vega MR, et al. Heart and skeletal muscle are targets of dengue virus infection. *Pediatr Infect Dis J*. 2010 March ; 29(3): 238–242.

3. Yacoub S, Wertheim H, Simmons C, Screaton G, Wills B. Cardiovascular manifestations of the emerging dengue pandemic. Nat. Rev. Cardiol 2014; 11, 335–345.

4. World Health Organization. Global strategy for dengue prevention and control 2012-2020. 2012.

5. Bravo L, Roque VG, Brett J, Dizon R, L'Azou M (2014).Epidemiology of dengue disease in the Philippines (2000–2011): A Systematic LiteratureReview. PLoSNegl Trop Dis 8(11): e3027

6. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. World Health Organization (WHO) and the Special Programme for Research and Training in Tropical Diseases (TDR), 2009.

7. Yacoub S, Wertheim H, SimmonsC, Screaton G, Wills B. Cardiovascular manifestations of the emerging dengue pandemic. Nat. Rev. Cardiol. 2014; 11, 335–345.

8. Kindermann I, Barth C, Mahfoud F, Ukena C, Lenski M, Yilmaz A, et al. Update on myocarditis. J. Am. Coll. Cardiol. 2012; 59, 779–792.

9. Yacoub S, Griffiths A, Chau TTH, Simmons CP, Wills B, Hien TT, Henein M, Farrar J. Cardiac function in Vietnamese patients with different dengue severity grades. Crit Care Med. 2012 February; 40(2): 477–483.

10. Lateef, A, Fisher, DA, Tambyah PA. Dengue and relative bradycardia. Emerging Infectious Diseases. Apr 2007; www.cdc.gov/eid Vol. 13, No. 4.

11. Wichmann D, Kularatne S, Ehrhardt S,Wijesinghe S, Brattig NW, Abel W. Cardiac involvement in dengue virus infections during the 2004/2005 dengue fever season in Sri Lanka. Southeast Asian J Trop Med Public Health 2009; 40: 727–730.
12. Wiwanitkit, Viroj. Dengue myocarditis, a rare but not fatal manifestation. Int J Cardiol.2006;112:122.

13. Shah, Ira.Dengue presenting as viral myocarditis. Dengue Bulletin. 2007; Vol 31.

14. Satarasinghe RL, Arultnithy K, Amerasena NL, Bulugahapitiya U, Sahayam DV. Asymptomatic myocardial involvement in acute dengue virus infection in a cohort of adult Sri Lankans admitted to a tertiary referral centre.Brit J Cardiol. 2007; 14: 171-173.

15. Gupta VK, Gadpayle AK. Subclinical cardiac involvement in dengue haemorrhagic fever. Journal, Indian Academy of Clinical Medicine.2010; Vol. 11, No. 2.

16. Yantie N, Gunawijaya E, Suradipa I, Gustawan I. Asymptomatic cardiac rhythm abnormality in children with dengue virus infection. Bali Medical Journal. 2016;5(2).
17. Wali JP, Biswas A, Chandra S, Malhotra A, Aggarwal P, Handa R, et al. Cardiac involvement in dengue haemorrhagic fever. Int J Cardiol. 1998; 64: 31-36.

18. La-Orkhun V, Supachokchaiwattana P, Lertsapcharoen P, Khongphatthanayothin A. Spectrum of cardiac rhythm abnormalities and heart rate variability during the convalescent stage of dengue virus infection: a Holter study. Ann. Trop. Paediatr 2001; 31, 123–128.

19. Hussain SBS, Kuswiyanto RB, Iwan J. Electrocardiogram profile in children with dengue infection at Dr. Hasan Sadikin General Hospital and Bandung City Hospital. Althea Medical Journal. 2016; 3(4):629–32.

20. Park KM. Pediatric Cardiology for Practitioners. 5th ed. Mosby, An imprint of Elsevier. 2008.

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