*Title:* **The Effect of Standard hydration therapy with Immunoglobulin colostrum versus Standard hydration therapy with Placebo in hospitalized patients with Dengue fever with warning signs: A Randomized, Double Blind, Placebo controlled trial.**

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*Abstract:*
**Background:** Dengue infection is a systematic and dynamic disease and has wide clinical spectrum of severe and non-severe forms. Resource allocation in dengue drug discovery is modest compared to other viral illnesses; a clinical drug candidate is yet to be identified. In several observational studies, immunoglobulin colostrum was hypothesized to prevent endothelial damage and affect cytokine production, which are relevant in the pathogenesis of dengue.

**Methods:** This study is a single-center, randomized, double blind, placebo-controlled trial of five days 150 mg immunoglobulin colostrum versus placebo in 46 Filipino adults with a positive NS1 rapid test presenting within 72 hours of fever onset. The outcomes were incidence and severity grade of dengue probable signs and warning signs, mean values and severity grade of complete blood count parameters, fever clearance time and length of hospital days.

**Results:** In this study supplementation of immunoglobulin colostrum versus placebo showed significant difference in the incidence of headache (*p* value =0.014), myalgia (*p* value = 0.016,0.013,0.029), arthralgia (*p* value = 0.02), retro-orbital pain (*p* value = 0.017), and mucosal bleeding (*p* value = 0.037, 0.028) as well as severity grade of headache (p value = 0.028), myalgia (p value 0.046, 0.01), arthralgia (*p* value = 0.013, 0.013, 0.034), retro-orbital pain (*p* value = 0.023), anorexia (*p* value = 0.04, 0.028), mucosal bleeding (*p* value = 0.37, 0.017), and abdominal pain (*p* value = 0.032). The severity grade of hemoglobin (*p* value = 0.044, 0.004, 0.014, 0.015), platelet count (*p* value =0.022), and wbc count (*p* value = 0.001) showed statistically significant difference. Fever clearance time showed statistically significant difference between group groups (*p* value = 0.01). Length of hospital stay has no statistical significant difference between group (*p* value = 0.2864).

**Conclusion:** Immunoglobulin colostrum is a relatively safe adjunct with standard hydration therapy in dengue fever with warning signs. It lowered fever clearance time and severity grade of headache, myalgia, arthralgia, anorexia, abdominal pain and mucosal bleeding. Incidence of headache, myalgia, arthralgia and mucosal bleeding was decreased with its use. Hemoconcentration, leucopenia, and thrombocytopenia severity grade was also lowered, however, it failed to show benefit in reducing hospital stay of patients.
*Key words:* Immunoglobulin colostrum (IgCo), Dengue Fever, Probable signs, Warning signs

## I. INTRODUCTION:

Dengue fever has been declared by the World Health Organization (WHO) to be the fastest spreading arthropod borne viral disease of the century.[1] It is caused by positive sense RNA virus of the *Flaviridae* family with four antigenically distinct serotype designated as Dengue viruses (DENV) serotype 1 to 4 that are predominantly transmitted through the mosquito vector *Aedes aegypti*.[2] Globally, it is estimated that 3.6 billion people in more than 100 tropical and sub-tropical countries are at risk for dengue, and making it as a leading cause of illness and death among affected population.[1,3] Among the tropical countries in the Western Pacific and Association of Southeast Asian Nations (ASEAN), Philippines ranked fourth with the highest incidence of dengue in recent years.[4] The increasing incidence of Dengue fever remains to be a problem, since its prevalence is evident throughout the year with a peak incidence during the months of June to September. The dengue case fatality rate in the Philippines from 2008 to 2012 was at 0.55%, wherein 3,195 deaths among 585,324 cases were reported.[5] The Department of Health (DOH) recorded an estimate of 78,808 Dengue cases from January to September 2015 with 233 mortalities. In Cordillera Administrative Region (CAR), the DOH Epidemiology Bureau reported 3,646 cases for 2015. At Saint Louis University-Hospital of the Sacred Heart Baguio City, the number of admitted cases from 2014, 2015 and 2016 were 204, 698, and 1194 and among these cases, the numbers of affected adults were 101, 394, and 389 respectively. Males were more commonly affected than females.

Dengue infection is a systematic and dynamic disease. It has a wide clinical spectrum that includes severe and non-severe forms of clinical manifestations. After the incubation period, the illness spectrum begins abruptly and will be followed by 3 phases: febrile, cirtical and recovery phase. The febrile phase usually lasts 2-7 days and usual symptoms of headache, body malaise, myalgia, arthralgia, retro-obrital pain, anorexia, nausea, vomiting, diarrhea, flushed skin, rash and laboratory tests shows leucopenia with or without thrombocytopenia. It is usually in this phase that NS1 antigen tests positive. Defervescence occurs on day 3-7 of illness, when the temperature drops to 37.5$^{o}$C to 38$^{o}$C or less and remains below this level. Around the time of defervescence, patients can either improve or deteriorate to progress to dengue with warning signs or severe dengue. The period of clinically significant plasma leakage usually lasts 24 to 48 hours, which is

known as the critical phase.[5] The critical phase follows the period of peak viremia, and usually shows rapid and complete reversal, suggesting it is likely to occur as a result of inflammatory mediators, rather than infection of the endothelium. Cytokines such as TNF-a, which are known to be elevated in the critical phase of dengue, are likely to be contributing factors. Dengue NS1, a soluble viral protein, has also been shown to disrupt the endothelial glycocalyx and thus contribute to vascular leak, although there appears to be discordance between the timing of NS1 antigenemia and occurrence of vascular leak. In addition, many inflammatory lipid mediators are elevated in acute dengue viral infection such as platelet activating factor (PAF) and leukotrienes. Furthermore, many other inflammatory mediators such as vascular endothelial growth factor and angiopoietin-2 have shown to be elevated in patients with dengue hemorrhagic fever, exerting their action in part by inducing the activity of phospholipases, which have diverse inflammatory effects including generation of PAF. Platelets also have shown to significantly contribute to endothelial dysfunction by production of IL-1B via activation of the NLRP3 inflammasome and also by inducing the production of inflammatory cytokines by monocytes.[6]

A gradual re-absorption of extravasated fluid from the intravascular to extravascular space by way of the lymphatics will take place in the next 48-72 hours. Hydration therapy remains to be the cornerstone of management for dengue.[5]

Since resource allocation in dengue drug discovery is modest compared to other viral illnesses, a clinical drug candidate is yet to be identified. The problem on resource allocation for developing new drugs for dengue fever was based on the demographics that the majority of affected population belongs to resource poor country where-in its buying power for a possible new anti-viral drug is questionable. The shift towards re-purposing existing drugs among reported and ongoing clinical trials for dengue is evident. The World Health Organization's International Clinical Trial Registry Platform includes a limited number of clinical trials for dengue that are mostly randomized, double-blinded, placebo-controlled trials.[7] Most of these drugs were utilized based on their proposed mechanism of action in dengue pathogenesis (Figure 1.1). [7]

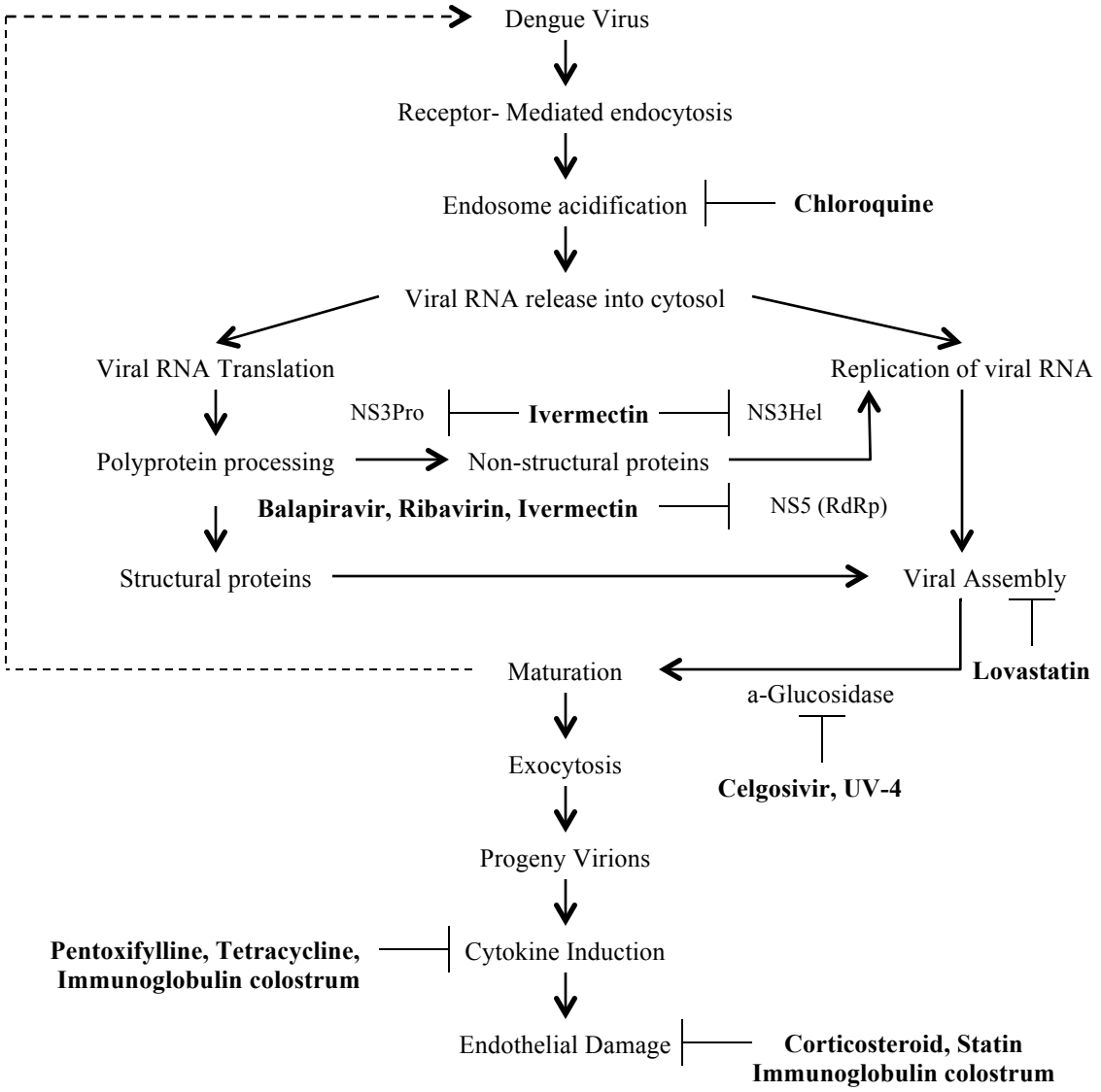


Figure 1.1 Dengue pathogenesis and drug targets.[7]

The dengue therapeutic clinical trials that were completed and reported since 2010 used the following drugs: chloroquine[8,9], prednisolone[10,11], lovastatin[12,13], balapiravir[14], and celgosivir[15]. The use of traditional herbal extracts[16-19] from *Cissampleos pareira* and *Carica papaya* were also explored[20]. The data from ongoing clinical trials on the use of Ivermectin, UV-4, Ribavirin, Zinc Bis-glycinate and Vitamin E for dengue will still be collated and reported. Other studies also explored the use of pentoxifylline[21]and tetracycline[22]. The mechanisms of action of the medications utilized in different dengue therapeutic clinical trials were geared towards the following in the

pathogenesis of dengue: endosome acidification inhibition (chloroquine); viral protein inhibition (Ivermectin, Balapiravir, and Ribavirin); viral assembly inhibition (Lovastatin); cytokine induction inhibition (Pentoxifylline and Tetracycline); stabilization of the vascular endothelium (Corticosteriod and Statin); and inhibition of host enzyme a-Glucosidase that aids virion assembly and maturation (Celgosivir and UV-4).[7]

The recent development of successful vaccine against dengue virus was complex and challenging since it must afford a simultaneous robust and durable protection against all four DENV serotypes[23,24]. Phase III trials of a live attenuated vaccine have revealed that the vaccine is not very effective against DENV-2[25,26]. This unexpected result, which underscores the need to explore other vaccine alternatives, has also renewed interest in fast-tracking drug discovery and development.[27]

This study investigated the possible clinical use of Immunoglobulin colostrum as an adjunct treatment for patients with dengue fever. The Immunoglobulin colostrum is 100% naturally derived dietary supplement obtained from bovine colostrum secreted during the first few days after calving. Bovine colostrum is an immune modulator. The immune modulation of bovine colostrum is attributed mainly to the bioactive components and factors present. These are responsible for neutralization of pathogen toxins, cytokine induction modification, and inhibition of reactive oxygen specie and nitric oxide. The Immunoglobuins, lactoferrin, lactoperoxidase, lysozyme, complement, glycoproteins and lipids present in bovine colostrum can exclude and neutralize infective agents and toxin that will modify the interaction of adhesion expressing pathogens with their target tissues, or block the adhesion receptors on the target tissue[28]. The oral preparation of Immunoglobulin colostrum in this study contained 150 mg of immunoglobulin G. In addition, transforming growth factor B and other growth factors present in bovine colostrum, were found to increase both IgG and especially IgA production in vitro[29]. A protein present in bovine colostrum called colostrinine, proline- rich polypeptide (PRP) protein shows a regulatory activity in cytokine (IFN, TNF-alpha, IL-6, IL-10) induction and possesses the ability to inhibit the overproduction of oxygen reactive species and nitric oxide.[30] This observation modulates its effect in vascular endothelium maintaining the vascular integrity. The proposed mechanism for its clinical utility in patients with dengue fever remains to be proven hence, this study was conceptualized.

## II. GOALS AND OBJECTIVES

*General Objective:* The study aimed at assessing the clinical efficacy of Immunoglobulin Colostrum in hospitalized patients with Dengue fever with warning signs, admitted at Saint Louis University- Hospital of the Sacred Heart from June 2017-September 2017.

*Specific Objectives:*

1. The study is aimed to determine the baseline characteristics of subjects with Immunoglobulin colostrum versus placebo.
2. The study is aimed to determine among subjects given with Immunoglobulin colostrum versus placebo, if there is a significant difference in the incidence and severity grade during hospital days one to five of:
    a. *Dengue Probable signs* (*Headache, Body malaise, Myalgia, Arthralgia, Retro-orbital pain, Anorexia, Nausea, Vomiting, Diarrhea, Rash)*
    b. *Dengue warning signs* (*Abdominal pain or tenderness, Persistent vomiting, Fluid accumulation, Mucosal bleeding, Lethargy or restlessness, Liver enlargement).*
    c. *Level of Complete blood count (Hemoglobin/Hematocrit level, Leukocyte count, Platelet count)*
3. The study is aimed to determine if there is a significant difference among subjects given with Immunoglobulin colostrum compared to placebo.
    a. Fever clearance time (in hours)
    b. Number of hospital stay (in days) from admission until discharge

## III. DESIGN

A randomized, double blind, placebo-controlled trial was done to investigate the effects of Immunoglobulin Colostrum therapy in the treatment of Dengue Fever with warning signs. This is a single center study conducted at Saint Louis University-Hospital of the Sacred Heart. The study was conducted to include cases of Dengue Fever with warning signs from June 2017 to September 2017.

*Research population:*

The sample size was computed using OpenEpi version 3.01 for comparing 2 means by Minn, Soe, using the study of Patiroglu[31], 2013 as reference wherein bovine colostrum group had a significantly lower infection severity score for viral upper respiratory infection than the placebo group after 1-week administration (p value=0.000). Total sample size of 14 was generated with 7 subjects in each treatment arm at 95% confidence interval. However, to account for dropout rate of 20% (based on the reference study), a total sample size of 17 was computed. Inclusion of more study participants was allowed until end of data collection with consideration for budget and resources which led to a total population of 46 patients, 23 in each study group.

The *Inclusion criteria* are as follows:

- 18- 60 years old
- Presented with less than or equal to 72 hours of febrile onset
- Fulfilled the clinical case definition for a Probable Dengue based on the Department of Health Revised Dengue Clinical Case Management Guidelines of 2011
    - Lives in or has a travel history to dengue-endemic area developing fever
    - Plus any *two* of the following: headache, body malaise, myalgia, arthralgia, retro-orbital pain, anorexia, nausea, vomiting, diarrhea, rash
    - *AND* Laboratory Test, at least CBC (leukopenia with or without thrombocytopenia) and/or dengue NS1 antigen test
- Fulfilled the clinical case definition for Dengue with warning signs based on the Department of Health Revised Dengue Clinical Case Management Guidelines of 2011
    - Abdominal pain or tenderness, persistent vomiting, clinical signs of fluid accumulation, mucosal bleeding, lethargy or restlessness, liver

enlargement, Laboratory test of an increase in Hematocrit and or decreasing platelet count

The *Exclusion criteria* are as follows:

- Patients who fulfilled the case definition for Severe Dengue base on the Department of Health Revised Dengue Clinical Case Management Guidelines of 2011
    - Severe plasma leakage leading to shock or fluid accumulation with respiratory distress
    - Severe bleeding
    - Severe organ impairment as evidenced by an elevated alanine aminotransferase (> 100 U/L), impaired consciousness, seizures, myocarditis, renal failure
- Known food or drug allergies
- Co-existing conditions such as:
    - Pregnancy, Obesity, Coronary artery disease, Cerebrovascular disease, Chronic Kidney Disease, Diabetes, Hypertension, Dyslipidemia, Bronchial Asthma, Chronic Obstructive Pulmonary Disease, Inflammatory Bowel Disease, Viral Hepatitis, Cancer, Lactose Intolerance, Drug and/or food allergies
- Previous history of Dengue fever
- Dengue IgG positive
- Received Dengue Vaccine
- Concomitant bacterial infection of the respiratory tract, gastrointestinal tract, genitourinary tract, central nervous system, or any focal infection pertaining to other organ systems
- Inability to provide an informed consent

The *Withdrawal criteria* are as follows:

- Progression to Severe Dengue base on the Department of Health Revised Dengue Clinical Case Management Guidelines of 2011

    - Severe plasma leakage leading to shock or fluid accumulation with respiratory distress
    - Severe bleeding
    - Severe organ impairment as evidenced by an elevated AST or ALT >100 U/L, impaired consciousness, seizures, myocarditis, renal failure
- Outcome severity grade 4 (life threatening) and/or 5 (causing death)
- Development of allergy to the medication as evidence by urticarial wheals/hives and development of severe bronchospasm

*Duration of the study:* 4 months

## IV. METHODOLOGY

The research protocol was reviewed and approved by the Research Ethical Committee of Saint Louis University prior to the conduct of the study. Prior to enrolment of patients, a written informed consent was obtained immediately upon admission at the room of choice of patients and to provide an adequate conducive environment for obtaining the consent of the prospective subjects who met the inclusion criteria.

A Double blind study with 1:1 random allocation of patients to either Immunoglobulin group/IgCo group (Group A; n = 23) or placebo group (Group B; n = 23) treatment groups was made. A computerized block randomization was done by a group of non-compensated emergency room nurses on duty. Group allocation was maintained in a sealed opaque envelope. Nurses, Clinical Pharmacist, Resident physicians, and Attending consultants involved in the study were blinded to the therapy administered to each patient. A group of non-compensated clinical Pharmacists not involved in the study opened sealed opaque envelopes prior to dispensing ten 15 g sachets of Immunoglobulin colostrum or visually identical placebo. The ward nurses not involve in the study opened the sealed opaque envelope to note what was given to the subjects and was recorded accordingly. The medication was prepared and administered by the same ward nurses by dissolving 15 g sachet powder solution in a 100 ml room temperature ($20^{o}$C) to lukewarm ($<50^{o}$C) distilled water and was taken by patients in an empty stomach twice a day, 30 minutes to two hours before breakfast and before bed time for five days. Whether the patient received Placebo or Immunoglobulin Colostrum was

not recorded in the chart, however, this was documented in a sealed enveloped attached to the patients chart and can be opened in case of progression to Severe Dengue during observation days and/or development of an immediate hypersensitivity reaction or drug allergy between 30 minutes to two hours observation period that is, after administration of the medication and prior to taking any meals and/or bed time.

Hydration therapy is the standard treatment for Dengue fever. Patients in both groups were hydrated orally and intravenously. A group of non-compensated Internal Medicine Resident physicians, who are at the same time, are the Medical Residents In Charge of the subjects, and are not involved in the study, computed the fluid hydration rate in milliliters per hour. Computation of fluids for hydration was based on the Department of Health Revised Dengue Clinical Case Management Guidelines of 2011 for calculation of oral hydrating fluid and/or intravenous fluid infusion. The type of fluid solution administered was standardized according to the recommended oral hydrating solution and/or intravenous fluid solution by the said guidelines. Adequacy of hydration was monitored and recorded accordingly using a hemodynamic assessment tool where in the following parameters was monitored and recorded daily: level of sensorium (clear and lucid, or restless and combative), capillary refill time (brisk- <2 secs, prolonged- > 2 sec); quality and characteristic of pulses (grade 0 – absent, grade +1 – diminished or decreased, grade +2 – normal, grade +3 – full pulse or slightly increased, grade +4 - bounding); palpation of extremities (warm, cool, cold and clammy); heart rate (beats per minute); blood pressure (mmHg); pulse pressure (mmHg); respiratory rate (cycles per minute); and urine output (cc/hr).

A standardized clinical assessment tool was utilized for assessing outcome parameters of the study. During the course of the subjects hospital stay, the same group of non-compensated Internal Medicine resident physicians not involved in the study recorded assessed outcome parameters in the standardized clinical assessment tool forms. The outcomes were recorded according to period of onset and duration, extent of causation if medication related (definitely related, probably related, possibly related, possibly not related, probably not related, definitely not related) and severity was graded according to the Common Terminology Criteria for Adverse Events System version 4.03 (CTCAE).

The clinically assessed outcome parameters were fever (axillary temperature $\geq$ 38.7$^{o}$C) clearance time in hours, probable dengue signs (*Headache, Body malaise, Myalgia, Arthralgia, Retro-orbital pain, Anorexia, Nausea, Vomiting, Diarrhea, Rash),* and dengue warning signs (*Abdominal pain or tenderness, Persistent vomiting, Fluid accumulation, Mucosal bleeding, Lethargy or restlessness, Liver enlargement).*

A blood sample for complete blood count, alanine aminotransferase, and serum creatinine was obtained as a base line measurement parameter. Complete blood count was monitored daily, analyzed using Coulter Beckman HMX analyzer, and values were recorded to note the improvement or progression of leucopenia (<5 x $10^9$/L), thrombocytopenia (<150 x $10^9$/L), and hemoconcentration (increased hematocrit of 20% from the baseline or values above the upper normal value for age and sex). The alanine aminotransferase (U/L) and serum creatinine (mg/dl) levels, although not routine in dengue management helped determine hepatic and renal function during the enrolment of the subjects in the study and assured that that subjects has not develop hepatic and renal impairments that signifies Severe dengue. The ALT and creatinine was measured, using Roche Cobas C311 analyzer. The laboratory investigations for myocarditis if suspected in subjects who developed chest pain and symptomatic bradycardia (decreased heart rate for age and sex) during the course of illness are 12-Lead electrocardiography, quantitative Troponin-I, and 2D- echocardiography. An Ultrasonography of the liver is made to subjects who has either an above normal liver span (in cms), and/or who test positive for fluid wave, and/or with severe abdominal pain (CTCAE grade 3) to document quantitative measurements of the liver size and/or presence of ascites.

Severity grade 4 and 5 outcomes, and/or progression to Severe dengue will necessitate termination of the administration of the medication and will be treated according to the Department of Health Revised Dengue Clinical Case Management Guidelines of 2011 for patients with Severe dengue requiring emergency treatment and urgent referral, such as patients with compensated shock, hypotensive shock and patients developing hemorrhagic complications leading to shock. Multi-disciplinary referrals will only be done if subjects developed outcome severity grade 4 and 5.

The subjects was assessed daily throughout their hospital stay and until *discharge criteria* are met such as:

- No fever for 48 hours (axillary temperature of less than <37.8$^{o}$C)
- Improvement of clinical status:
    - general well being (visual analog scale)
    - appetite (fair, good, excellent)
    - hemodynamic status
        - clear and lucid level of sensorium
        - brisk capillary refill time (<2 secs)
        - normal pulses (grade +2)
        - warm extremities
        - normal heart rate for age and sex
        - normal blood pressure for age and sex
        - normal pulse pressure (30-40 mmHg)
        - adequate urine output (> 30cc/hr)
        - normal respiratory rate for age and sex
- Increasing trend of platelet count (x$10^{9}$/L)
- Stable hematocrit without intravenous fluid (normal for age and sex)

Results was tallied and interpreted accordingly after the appropriate sample size was achieved.

## V. SAFETY OF REASEARCH SUBJECT/PARTICIPANTS

The safety of the subjects was a primary consideration in this study. Since dengue fever with warning signs can evolve during the course of illness to severe dengue, the need to grade severity of outcomes for additional medical interventions was necessary. The use of other medical management other than hydration in dengue fever remains anecdotal; hence, the study utilized certain medications for symptomatic treatment of the subjects.

Severity grade 2 or 3 headache, body malaise, myalgia, arthralgia, and retro-orbital pain warranted use of analgesics. The analgesic of choice of either Paracetamol 500 mg one tablet every four hours or Orphenadrine + Paracetamol 35mg/450 mg one

tablet three times a day as needed for pain was given. Appetite enhancers and/or Enteral or Parenteral nutrition indicated for severity grade 2 or 3 anorexia. An anti-emetic, Metoclopramide 10 mg IV was given every eight hours as needed for nausea and vomiting severity grade 2 or 3 or round the clock administration for persistent vomiting regardless of severity grade. Diarrhea warranted volume for volume replacement of fluids either of oral or intravenous route regardless of severity grade. Rash with accompanying symptom such as pruritus regardless of severity grade, used an anti-histamine of either Cetirizine 5-10 mg 1 tab once to twice a day or Diphenhydramine 50 mg IV every 8 hours as need for pruritus. An abdominal pain severity grade 2-3 necessitated further investigation of the characteristic of pain, for burning sensation, a proton pump inhibitor, Omeprazole 20-40 mg 1 tab 30 mins to one hour prior to breakfast was given, for bloatedness, Domperidone 10 mg 1 tab three times a day for five days was given, for carampy or spastic abdominal pain, an anti-spasmodic, HNBB 10 mg 1 tab every 8 hours was given, and for vague abdominal pain, an analgesic was given (Paracetamol or Orphenadrine). Fluid accumulation severity grade 2-3 will require use of diuretic. Administration of Furosemide 20-80 mg tab/IV and adjustment of fluid hydration can be done. Subspecialty referrals will be necessary for severity grade ≥ 3 fluid accumulation that will indicate interventions to do therapeutic and/or diagnostic thoracentesis, paracentesis, or pericardiocentesis. Mucosal bleeding severity grade 2 will necessitate medical intervention such as packing, cauterization or use of topical vasoconstrictors. In severity grade 3 mucosal bleeding, indication for transfusion of blood products to subjects will be based on the recommendations of the Department of Health Revised Dengue Clinical Case Management Guidelines of 2011. Procedures such as radiologic, endoscopic or operative intervention for mucosal bleeding will necessitate subspecialty referral. Presence of lethargy or restlessness will necessitate subspecialty referral regardless of severity grade. Liver enlargement severity grade 2-3 will need subspecialty referral. The study will be terminated for subjects developing severity grade 4-5 outcome parameters, impaired consciousness, seizure, elevated alanine aminotransferase level (>3x ULN or ALT >100 U/L), myocarditis, and renal failure and will be recorded as drop out.

This study utilized Immunoglobulin Colostrum skim milk powder preparation. This is a 100% naturally derived product from bovine colostrum. Research has shown that bovine colostrum is safe and easily assimilable by humans. There have been no contraindications and major side effects reported through years of human consumption of bovine colostrum. [32] No interactions to other medications have also been noted with Immunoglobulin colostrum usage.

However, known allergy to cows milk and derived product may hinder the use of this preparation. In case of development of allergy symptoms such as urticarial wheals/hives and severe bronchospasm, immediate discontinuation of the medication will be done. Subcutaneous or intramuscular injection of Epinephrine 1:1000 (1mg/ml) dilution at intervals of 5-20 minutes will be administered until resolution of symptoms is evident. Diphenhydramine 50-100 mg intravenous or intramuscular injection and/or intravenous Methylprednisolone 0.5-1mg/kg can also be given if symptoms are persistent despite epinephrine use. The adverse event was recorded according to period of onset and duration, extent of causation, and graded according to the CTCAE version 4.03.

Visually identical placebo was used. The placebo is a pure starch base product that is not under trial. It is a carbohydrate polymer that is easily digested. It is tasteless, colorless when dissolved and odorless.

Follow-up was provided for the participants in the duration of their hospital stay. Adverse events will still be monitored during the research duration up to patient's discharge and will be treated accordingly.

## VI. DATA MANAGEMENT AND STATISTICAL ANALYSIS

*Data Management:* Data obtained was immediately recorded in a laboratory logbook. The researcher administered data assessment work sheet. Responses were coded to make the data suitable for analysis. Microsoft Excel was the main data management tool in the study. Two separate individuals creating two separate databases, which were compared, entered data into the computer.

*Data analyses:* The data yield was analyzed using the SPSS version 19 software. For the descriptive statistics, continuous variables such as age, length of hospital stay, fever clearance time, and blood parameters were measured in terms of means and standard deviation while categorical variables such as incidence and severity grade of dengue probable signs and warning signs were measured in terms of frequencies and percentages. To obtain statistical inference between treatment group (Igco group) and control group (placebo), 2-independent sample t-test for continuous variables and Chi- square test for categorical variables were utilized. Statistically significant difference is noted if *p* value is <0.05 at 95% Confidence interval.

## VII. ETHICAL CONSIDERATIONS

The research protocol was reviewed and approved by the Research Ethics Committee of Saint Louis University prior to the conduct of the study. The review considered both scientific and ethical aspects of the research. Permission to conduct the research was asked from the Administration Office and Training Office of Saint Louis University Hospital of the Sacred Heart thru the Board of Research of the same institution.

The informed consent was obtained immediately at the room of choice of subjects prior to the enrollment in the study, which is during the subject's admission day. The researcher personally obtained the informed consent through constructive dialogue between the prospective subjects. All prospective subjects were of legal age (18 years old and above) with full cognitive ability in providing legally binding informed consent. Substitution for providing an informed consent, such in cases of impaired cognitive ability, consent was only obtained thru the authorization of the legal next of kin or a representative provided that they are of legal age. The content of the informed consent was presented and explained in a sequential manner elaborating the benefits and risks involved in the study. The benefits among subjects would include less hospital expenses since prospective subjects was given the medication free of charge and the possible therapeutic benefits of the Immunoglobulin Colostrum to booster the immune response of the patient providing lesser illness severity. The risk was very minimal. The risks include

developing hypersensitivity reaction and symptoms of lactose intolerance. However, this would be addressed accordingly as stated above.

The investigator provided sufficient time for the prospective subject to explain in his/her own words the level of understanding of the research and delayed consent was provided in cases where the prospective subject wish to confide opinions of his/her significant others or relatives regarding the participation in the study. In this way, prospective subjects was provided enlightened decision without coercion or undue influence. If the individual was uncomfortable or anxious about participating in the research they were instructed to take time to read and review the informed consent form, before deciding whether or not to participate in the research. The investigator had the responsibility to document the informed consent. The prospective subject had the right to withdraw during the duration of the study. The signing of the informed consent was done with the investigator and consenting subjects presence. Complete anonymity of the research participants was observed, as the participants was not asked to write their names or any identifying marks on the standardized clinical assessment tool. A coding system was used to remove some or all-direct identifiers pertaining to prospect subjects. The researcher employed secured storage of files and disposal of documents after the completion of the research. Only the researcher would know specific information regarding the participant. Thus, specific information could not be linked to specific individuals. Access to the data was limited only to the researcher and research adviser.

## VIII. RESULTS:

The study was conducted among patients admitted with dengue fever with warning signs at Saint Louis University-Hospital of the Sacred Heart from June to October 2017. There were 46 study population from the start until the end of the study (n=23 IgCo group, n=23 Placebo group).

**Table 1.** Baseline Characteristics between IgCo Group versus Placebo Group

| Baseline Characteristics | IgCo Group (n=23) | | | Placebo Group (n=23) | | | *p* value |
|---|---|---|---|---|---|---|---|
| | Mean ± SD | Frequency (%) | (%) | Mean ± SD | Frequency (%) | (%) | |
| **Demographic Variables** | | | | | | | |
| Age (years) | 33.09 ± 13.43 | | | 32.26 ± 11. 90 | | | 0.83 |
| Sex | | Male: 10<br>Female: 13 | 43.48<br>56.52 | | Male: 12<br>Female: 11 | 52.17<br>47.82 | 0.55 |
| History of travel | | 11 | 47.82 | | 11 | 47.82 | 0.77 |
| **Clinical Variables** | | | | | | | |
| Fever Onset (hours) | 60.53 ± 15.97 | | | 32.26 ± 11. 90 | | | 0.7 |
| DNS1Ag | | 23 | 100 | | 23 | 100 | - |
| Dengue IgM | | 5 | 21.74 | | 8 | 34.78 | 0.33 |
| Hemoglobin (g/L) | 151.57 ± 14.31 | | | 149.57 ± 18.44 | | | 0.24 |
| Hematocrit | 0.44 ± 0.05 | | | 0.45 ± 0.05 | | | 0.54 |
| WBC ($x10^9$/L) | 4.69 ± 2.41 | | | 4.50 ± 1.90 | | | 0.77 |
| Platelet ($x1^9$/L) | 166.7 ± 50.24 | | | 175.48 ± 61.30 | | | 0.36 |
| Creatinine (mg/dL) | 0.84 ± 0.18 | | | 0.83 ± 0.16 | | | 0.59 |
| ALT (U/L) | 49.3 ± 25.52 | | | 49.88 ± 22.14 | | | 0.51 |
| **Probable Signs** | | | | | | | |
| Headache | | 18 | 78.26 | | 16 | 69.57 | 0.50 |
| Body malaise | | 23 | 100 | | 23 | 100 | - |
| Myalgia | | 13 | 56.52 | | 15 | 65.22 | 0.55 |
| Arthralgia | | 21 | 91.30 | | 22 | 95.65 | 0.55 |
| Retro-orbital pain | | 13 | 56.52 | | 18 | 78.26 | 0.12 |
| Anorexia | | 7 | 30.43 | | 10 | 43.48 | 0.36 |
| Nausea | | 6 | 26.09 | | 5 | 21.74 | 0.73 |
| Vomiting | | 2 | 8.70 | | 2 | 8.70 | 0.5 |
| Diarrhea | | 3 | 13.04 | | 2 | 8.70 | 0.64 |
| Rash | | 8 | 34.78 | | 9 | 39.13 | 0.76 |
| **Warning Sign** | | | | | | | |
| Abdominal pain | | 11 | 47.83 | | 12 | 52.17 | 0.77 |
| Persistent vomiting | | 0 | 0 | | 0 | 0 | - |
| Fluid retention | | 0 | 0 | | 0 | 0 | - |
| Mucosal bleeding | | 2 | 8.70 | | 2 | 8.70 | 0.5 |
| Lethargy | | 0 | 0 | | 0 | 0 | - |
| Liver enlargement | | 0 | 0 | | 0 | 0 | - |

Table 1 shows the baseline characteristics between IgCo and placebo group in terms of demographic variables, clinical variables, probable signs and warning signs of dengue fever. The mean age for IgCo and placebo group was 33 years and 32 years respectively. For gender distribution, there were 43.48% males and 56.52% females in the IgCo group while there were 52.12% males and 47.82% females in the placebo group. For both study groups, 47.82% had previous history of travel. The mean hours of onset of

fever from the time of enrolment was 60 hours in the IgCo group versus 32 hours in the placebo group. All tested positive for DNS1Ag with only 21.74% and 34.78% Dengue IgM positive for IgCo and placebo group respectively. Mean hemoglobin concentration (g/L) of 151.57 g/L and 149.57 g/L, with mean hematocrit of 0.44 and 0.45 for IgCo and Placebo group respectively. White blood cell count ($x10^9$/L) for both study group showed leukopenia at 4.69 and 4.50. Platelet count ($x1^9$/L) was normal for both study group at 166 $x1^9$/L and at 175 $x1^9$/L. Baseline alanine aminotransferase levels were slightly elevated by 1.2x from the upper normal limit at 49.3 U/L and 49.88 U/L for both groups. Normal creatinine level at 0.84 mg/dl and 0.83 mg/dl. The most common (100%) Probable dengue sign for both study groups is body malaise. In the IgCo group, the probable dengue signs in decreasing order of frequency are: arthralgia (91.3%), headache (78.26%), myalgia (56.52%), retro-orbital pain (56.62%), rash (34.78%), anorexia (30.43%), nausea (26.09%), diarrhea (13.04%) and vomiting (8.7%). In the placebo group, the probable dengue signs in decreasing order of frequency are: arthralgia (95.65%), retro-orbital pain (78.26%), headache (69.57%), myalgia (65.22%), anorexia (43.48%), rash (39.13%), nausea (21.74%), diarrhea (8.7%) and vomiting (8.7%). The most frequent warning sign for both study groups is abdominal pain noted at 47.83% in the IgCo group and 52.17% in the placebo group. Mucosal bleeding had equal frequency at 8.7% in both study groups. There were no noted persistent vomiting, fluid retention, lethargy, and liver enlargement at the start of the study. In all baseline characteristics, no significant difference exists between the IgCo group and placebo group.

**Table 2-A.** *P* values of the Incidence and Severity Grade of Dengue Probable signs from observation days one to five.

| Probable | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| signs | Incidence | SG | Incidence | SG | Incidence | SG | Incidence | SG | Incidence | SG |
| **Headache** | 0.502 | 0.646 | 1 | 0.162[b] | 0.552 | 0.549[b] | 0.014[b] | 0.007[b] | 0.161[b] | 0.28[b] |
| **Body Malaise** | -[a] | 0.681[b] | -[a] | 0.148[b] | -[a] | 0.139[b] | -[a] | 0.292[b] | -[a] | 0.558 |
| **Myalgia** | 0.546 | 0.823[b] | 0.134 | 0.311[b] | 0.016 | 0.046[b] | 0.013[b] | -[a] | 0.029[b] | 0.01[b] |
| **Arthralgia** | 0.547[b] | 0.759[b] | 0.547[b] | 0.759[b] | 0.127 | 0.013 | 0.02[b] | 0.013 | 0.104 | 0.045[b] |
| **Retro-orbital pain** | 0.116 | 0.092[b] | 0.017 | 0.023[b] | 0.174 | 0.079[b] | 0.104[b] | 0.077[b] | 0.161[b] | 0.077[b] |
| **Anorexia** | 0.359 | 0.359 | 0.061 | 0.06 | 0.08 | 0.084 | 0.06 | 0.04[b] | 0.07[b] | 0.028[b] |
| **Vomiting** | 1 | 1 | 1 | 1 | 0.148 | 0.091 | 0.312 | -[a] | 0.475 | -[a] |
| **Diarrhea** | 0.635[b] | 0.635[b] | 0.635[b] | 0.635[b] | 0.235[b] | 0.235[b] | 0.235[b] | -[a] | 0.475 | -[a] |
| **Rash** | 0.76 | 0.946[b] | 0.765 | 0.949[b] | 0.536 | 0.821[b] | 0.478[b] | 0.227[b] | 0.654[b] | 0.463[b] |

a- not estimable since the variable is a constant
b- Chi square test used likelihood ratio

Table 2-A shows the *p* values of the incidence and severity grade of dengue probable signs between IgCo group and placebo group during observation days one to five. There was significant difference (*p* value = 0.014) in the incidence of headache during observation day four and severity grade of headache during observation days four and five (*p* value = 0.007, 0.028) between IgCo and placebo group. The incidence of myalgia between IgCo group and placebo group during days three to five showed significant difference (*p* value = 0.016, 0.013, 0.029). The severity grade of myalgia during days three to five showed significant difference between the study groups (*p* value = 0.046, -, 0.01). There was a significant difference (*p* value = 0.02) in the incidence of arthralgia during observation day four between IgCo and placebo group, where as, the severity grade of arthralgia showed significant difference (*p* value = 0.013, 0.013, 0.034) during observation days three to five. The incidence and severity grade of the retro-orbital pain showed significant difference (*p* value = 0.017, 0.023) during observation day two. There was significant difference (*p* value = 0.04, 0.028) in the severity grade of anorexia during days four and five between IgCo and placebo group. Among the probable signs of dengue, there was no significant difference observed during days one to five in the incidence and severity grade of body malaise, vomiting, diarrhea and rash between IgCo and placebo group.

**Table 2-B.** *P* values of the Incidence and Severity Grade of Dengue Warning signs from observation days one to five.

| **Warning signs** | **Day 1** | | **Day 2** | | **Day 3** | | **Day 4** | | **Day 5** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Incidence | SG | Incidence | SG | Incidence | SG | Incidence | SG | Incidence | SG |
| **Abdominal pain** | 0.768 | 0.885[b] | 0.375 | 0.101[b] | 0.375 | 0.378[b] | 0.0193[b] | 0.170 | 0.093[b] | 0.032[b] |
| **Persistent Vomiting** | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] | 0.235[b] | -[a] | 0.710[b] | -[a] |
| **Fluid accumulation** | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] |
| **Mucosal bleeding** | 1 | 1 | 1 | 1 | 0.037[b] | 0.037[b] | 0.028[b] | 0.017[b] | 0.798[b] | 0.576[b] |
| **Lethargy** | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] |
| **Liver Enlargement** | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] | -[a] |

a- not estimable since the variable is a constant
b- Chi square test used likelihood ratio

Table 2-B shows the p values of the incidence and severity grade of Dengue warning sings from observation days one to five between IgCo and placebo group. There was a significant difference (*p* value = 0.032) in the severity grade of abdominal pain between IgCo and placebo group during observation day five. There was a significant difference in the incidence (*p* value = 0.037, 0.028) and severity grade (*p* value = 0.37, 0.017) of mucosal bleeding between IgCo and placebo group during observation days three and four. Incidence of persistent vomiting during observation days four and five showed no significant difference between the study groups.

**Table 2-C.** *P* values for the Mean and Severity Grade of Complete Blood Count Parameters from observation days one to five.

| Parameter | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean[a] | SG[b] | Mean[a] | SG[b] | Mean[a] | SG[b] | Mean[a] | SG[b] | Mean[a] | SG[b] |
| Hemoglobin | 0.683 | 0.044 | 0.617 | 0.349 | 0.465 | 0.004 | 0.699 | 0.014 | 0.222 | 0.015 |
| Hematocrit | 0.524 | - | 0.919 | - | 0.543 | - | 0.448 | - | 0.248 | - |
| WBC count | 0.725 | 0.654 | 0.916 | 0.613 | 0.930 | 0.698 | 0.659 | 0.08 | 0.254 | 0.001 |
| Platelet Count | 0.598 | 0.486 | 0.521 | 0.09 | 0.046 | 0.101 | 0.095 | 0.643 | 0.072 | 0.022 |

*a- chi-square; b- t-test

Table 2-C shows the p values for mean and severity grade of the parameters for complete blood count from observation days one to five between IgCo and placebo group. The mean values of the complete blood count parameters were all comparable on the five time points except on observation day three where in the platelet count between IgCo and placebo group revealed significant difference (*p* value = 0.046). The severity grade of the hemoglobin values showed significant difference between groups on days one, three, four and five with the following *p* values 0.044, 0.004, 0.014, and 0.015 respectively. The platelet count and white blood cell count showed significant difference (p = 0.022, 0.001) in severity grade between study groups on observation day five.

**Table 3.** Fever clearance time (hours) and In-hospital stay (days) among IgCo Group versus Placebo Group

| Parameters | IgCo Group Mean± SD | Placebo Group Mean± SD | *p* value |
|---|---|---|---|
| Fever Clearance Time (hr) | 98.52 ± 19.07 | 115.13 ± 23.13 | 0.01 |
| Length of Hospital Stay (days) | 4.96 ± 0.98 | 5.30 ± 1.15 | 0.2864 |

Table 3 shows fever clearance time in hours and number of in-hospital stay in days among IgCo Group versus placebo Group. Fever clearance time was shorter in IgCo group at 98.52 hours compared to the placebo group at 115.13 hours. Fever clearance time showed statistically significant between the two groups ($p$ value = 0.01). The length of hospital stay in days is noted at 4.96 and 5.30 days for IgCo and placebo group respectively, which is not statistically significant ($p$ value = 0.2864).

## IX. DISCUSSION:

Several studies in the World Health Organization's International Clinical Trial Registry Platform failed to show proven and effective treatment for dengue other than fluid resuscitation or hydration.[7] In several observational studies, immunoglobulin colostrum was hypothesized to prevent endothelial damage and affect cytokine production, which are relevant in the pathogenesis of dengue.[22] In this randomized, double-blind, placebo-controlled trial among adults with dengue fever with warning signs, the study found that immunoglobulin colostrum was safe and well tolerated. Specifically, there was no evidence of severe adverse events (severity grade four or five) that warranted discontinuation of the medication among the study groups.

The population for this study was mostly of the middle-aged group with mean age between 32-33 years, equal gender proportion, no previous dengue infection, co-morbidities, stable renal and hepatic function, and with febrile onset between 32-60 hours prior to admission. Among the probable signs, the most common was body malaise followed in decreasing frequency of arthralgia, headache and retro-orbital pain. The most common warning sign was abdominal pain. Development of severe dengue among the study population was not evident. Studies have shown that existing co-morbidities among dengue patients were not factors that contribute to dengue disease and shown no correlation can be made between the clinical profile of the subjects to their risk for morbidity and mortality[33], however, retrospective analysis of dengue fever case management and frequency of co-morbidities associated deaths showed 60% had at least one co-morbid condition which are diabetes mellitus, hypertension or both[34]. Safety and efficacy of immunoglobulin colostrum among population affected with the disease with

co-morbid conditions remains to be proven and if supplementation with immunoglobulin colostrum will decrease the incidence for the development of severe dengue and in decreasing morbidity and mortality.

The clinical and laboratory outcome measurements were used to determine if supplementation of immunoglobulin colostrum with standard hydration therapy in patients with dengue fever was effective. These were compared and analyzed using incidence and severity grade of dengue probable signs and warning signs and level of complete blood count parameters. Fever clearance time and duration of hospital stay were also determined and compared between groups in this study.

Among patients who received immunoglobulin colostrum, the overall severity grade of probable signs and warning signs were lower compared to placebo in five time points of observation. The incidence of headache, myalgia, and arthralgia were reduced significantly during administration of the immunoglobulin colostrum on day four, and continuous significant reduction of the incidence of myalgia on latter observation period. Severity grade of headache, myalgia, arthralgia and anorexia were significantly reduced on the latter hospital days, and early severity grade reduction was noted in patients experiencing myalgia. Although the severity grade of the retro-orbital pain was significantly reduced on an earlier observation day, it failed to show benefit in reducing the severity grade of the said outcome on succeeding hospital days. It was also observed that immunoglobulin colostrum has no benefit in lowering the incidence and severity grade of body malaise, vomiting, diarrhea and rash. Among the warning signs, immunoglobulin colostrum proved to be beneficial in lowering the incidence and severity grade of mucosal bleeding on latter hospital days, as well as the severity grade of abdominal pain, however failed to show benefit in lowering the incidence of abdominal pain. There were no occurrence of lethargy and liver enlargement among both study groups, hence, the clinical use of immunoglobulin colostrum among patients with dengue fever with the said warning signs remains to be proven.

The causal relationship between proinflammatory cytokines, such as IL-6, IL1-B and TNF are believed to cause the majority of symptoms, such as fever, malaise, and coagulopathies associated with infections.[22] In another study, serum expression level of 36 cytokines were determined and was found out that GRO-a and IP-10 expression levels during the acute phase of dengue may serve as warning sign of infection severity.[35] Indeed, the degree of imbalance between such cytokines and their anti-inflammatory counterparts may be the primary prognostic indicator of disease outcome.[22] In these study, cytokine/chemokine determination was not done. The existing relationship of immunoglobulin colostrum and level of cytokine among patients with dengue fever remains to be proven despite beneficial effect of decreasing severity grade and incidence of some of the probable signs and warning signs for dengue. Existing clinical trials also failed to determine beneficial effect of trial medications in reducing incidence and severity grade of the clinical variables of probable signs and warning signs for dengue.[7]

Endothelial dysfunction is established by 72 hours[12-13] and the possibility of immunoglobulin colostrum in preventing endothelial damage leading to vascular leakage still remains to be proven. Despite commencement of supplementation of immunoglobulin colostrum during the early febrile phase of dengue fever in this study, supplementation of immunoglobulin colostrum failed to demonstrate reduction of disease severity and incidence of fluid accumulation, because there was no demonstrable occurrence of the said warning sign in both of the study groups. Therapeutic effect of several drugs under the World Health Organization's International Clinical Trial Registry Platform also failed to show benefit on fluid accumulation in dengue fever pathogenesis.[7]

Clinical laboratory endpoints using complete blood count parameters such as hemoglobin, hematocrit, wbc count and platelet count were analyzed for mean values. The course of the disease, there are major shifts within cellular component in the blood.[36] Common laboratory findings in patients with dengue are thrombocytopenia in 100% of cases, leucopenia in 52.8%, raised hematocrit in 50% and associated with overall mortality of 2.88%.[37] In this study, mean values were all comparable from baseline until the end of the observation days. Mean values during observation time points showed that the hemoglobin, hematocrit value and white blood cell count were at most similar for

both groups. It was only on the third observation day that there was clinical significant difference of mean platelet value between the study groups, however failed to show significant difference of the mean values over the succeeding days. Therefore, use of immunoglobulin colostrum in increasing platelet count in patients with dengue needs further investigation. However, decreased severity grades of hemoconcentration, thrombocytopenia and leucopenia for patients given with immunoglobulin colostrum were observed in this study over succeeding hospital days. Immunoglobulin colostrum in these study proved to be beneficial in improving the severity grade of patients' hemoconcentration, thrombocytopenia and leucopenia.

Fever clearance time improved with supplementation of immunoglobulin colostrum among patients with dengue fever with warning signs with a mean difference of 16.61 hours compared to placebo. The febrile phase of dengue usually occurs at three to five days duration, however, can occur for seven days in some instances.[3,7] Since immunoglobulin colostrum modulates cytokine induction and thus alter cytokine febrile response[28,30,31] supplementation with IgCo can shorten the course of the febrile phase among patients with dengue fever. Although the fever clearance time was shortened, the duration of in-hospital stay among dengue patients with warning signs who received twice a day dosing of the immunoglobulin colostrum for five days was not reduced. The average length of in- hospital stay was five days with or without supplementation of immunoglobulin colostrum.

## X. LIMITATIONS OF THE STUDY:

The study is a single-center study that has inherent limitations, since in this type of study the numbers of participants are not significantly large to make generalization to its applicability in real life. The selection of subjects was specific only to those who has no co-morbid conditions and to those who had first episode of dengue infection limiting its applicability in patients without existing co-morbid conditions and who had first dengue infection. The duration of conducting the study was only limited to four months duration. Testing for DENV serotype and viremia quantification were not done in this study.

## XI. CONCLUSION:

Immunoglobulin colostrum serves as relatively safe adjunct with standard hydration therapy in patients with dengue fever with warning signs in lowering fever clearance time as well as in lowering the severity grade of probable dengue signs and warning signs which are headache, myalgia, arthralgia, anorexia, abdominal pain and mucosal bleeding during the latter observation days. Incidence of headache, myalgia, arthralgia and mucosal bleeding was decreased with the use of immunoglobulin colostrum. Hemoconcentration, leucopenia, and thrombocytopenia severity grade was lower with immunoglobulin colostrum supplementation; however in this study it failed to show benefit in reducing hospital stay of patients with dengue fever with warning signs.

## XII. RECOMMENDATIONS:

The effect of immunoglobulin colostrum with standard hydration therapy for patients with dengue fever should be evaluated on a larger scale. A multi-center clinical study is recommended. Since the study was limited to patients with no-comorbidities and with first episode of dengue, it is recommended that use of immunoglobulin colostrum in individuals with recurrence of dengue and with co-morbidities should be evaluated if severity of the illness will be significantly affected. The frequency and duration of administration of the immunoglobulin colostrum should also be evaluated for its effect on future studies. Correlation between the peak viremia of dengue and use of immunoglobulin should also be made since there is a possibility that even initiating therapy within the timeframe of 72 hours is too late to modulate the disease pathway since peak viremia occurs at early stages of the disease. Therapeutic development for dengue should focus more on identifying chemophraphylactic agents that could be used in those at most risk of severe disease, and also in optimizing supportive care for those who will develop severe disease.

## XIII. REFERENCES:


1. Gubler DJ. The economic burden of dengue. Am J Trop Med Hyg. 2012;86:743–744.
2. Pierson TC, Diamond MS. Flaviviruses. In: Knipe DM, Howley PM, editors. Fields virology. 6th ed. Philadelphia (PA): Wolters Kluwer and Lippincott Williams & Wilkins; 2013. p. 747–794.
3. World Health Organization: Western Pacific Region (2008) Chapter 7. Priority communicable diseases. In: Health in Asia and the Pacific. Available: http:// www.wpro.who.int/health_research/documents/dhs_hr_health_in_asia_and_ the_pacific_12_chapter_7_priority_communicable_diseases.pdf. Accessed 24 March, 2017.
4. Yboa, B., & Labrague L. (2013). Dengue Knowledge and Preventive Practices among Rural Residents in Samar Province, Philippines. *American Journal of Public Health Research*, 1 (2), 47-52.
5. Department of Heatlh. (2012). Revised Dengue Clinical Case Management Guidelines 2011. *Administrative Order No. 2012-0006,* 1-27.
6. Malavigne GN, et al, Pathogenesis of vascular leakage in dengue virus infection, Immunology 2017
7. International Clinical Trials Registry Platform of the World Health Organization URL: apps.who.int/trialsearch/
8. Borges MC, Castro AL, Da Fonseca BAL. Chloroquine use improves dengue-related symptoms. Mem Inst Oswaldo Cruz. 2013;108:596–599.
9. Tricou V, Minh NN, Van TP, et al. A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. PLoS Negl Trop Dis. 2010;4:e785
10. Nguyen THT, Nguyen THQ, Vu TT, et al. Corticosteroids for dengue – why don't they work? PLoS Negl Trop Dis. 2013;7:e2592.
11. Tam DTH, Ngoc TV, Tien NTH, et al. Effects of short-course oral corticosteroid therapy in early dengue infection in Vietnamese patients: a randomized, placebo-controlled trial. Clin Infectious Dis. 2012;55:1216–1224
12. Whitehorn J, Chau NVV, Truong NT, et al. Lovastatin for adult patients with dengue: protocol for a randomised controlled trial. BMC Trials. 2012;13:203.
13. Whitehorn J, Nguyen CVV, Khanh LP, et al. Lovastatin for the treatment of adult patients with dengue: a randomized, double-blind, placebo-controlled trial. Clin Infect Dis. 2015. doi:10.1093/cid/civ949.
14. Nguyen NM, Tran CNB, Phung LK, et al. A randomized, double-blind placebo controlled trial of balapiravir, a polymerase inhibitor, in adult dengue patients. J Infect Dis. 2013;207:1442–1450.
15. Low JG, Sung C, Wijaya L, et al. Efficacy and safety of celgosivir in patients with dengue fever (CELADEN): a phase 1b, randomized, double-blind, placebo-controlled, proof-of-concept trial. Lancet Infect Dis. 2014;14(8):706–715.
16. Tang LIC, Ling APK, Koh RY, et al. Screening of anti-dengue activity in methanolic extracts of medicinal plants. BMC Comp Alt Med. 2012;12:3
17. Lee SH, Tang YQ, Rathkrishnan A, et al. Effect of cocktail of four local Malaysian medicinal plants (*Phyllanthus spp*.) Against Dengue Virus 2. BMC Comp Alt Med. 2013;13:192.
18. Sarala N, Paknikar SS. Papaya extract to treat dengue: A novel therapeutic option? Ann Med Health Sci Res. 2014;4:320–324.
19. Sood R, Raut R, Tyagi P, et al. *Cissampelos pariera* Linn: Natural source of potent antiviral activity against all four dengue virus serotypes. PLoS Negl Trop Dis. 2015;9:e0004255
20. Dengue and dengue haemorrhagic fever. WHO Factsheet No117. 2014. Available from: www.who.int/mediacentre/factsheets/fs117/en/
21. Salgado D, Zabaleta TE, Hatch S, et al. Use of pentoxifylline in treatment of children with dengue hemorrhagic fever. Pediatric Infect Dis J. 2012;31:771–773.
22. Castro JE, Vado-Solis I, Perez-Osorio C, et al. Modulation of cytokine and cytokine receptor/antagonist by treatment with doxycycline and tetracycline in patients with dengue fever. Clin Dev Immunol. 2011. Article ID 370872. doi:10.1155/2011/370872
23. Swaminathan S, Batra G, Khanna N. Dengue vaccines: state of the art. Expert Opin Therap Patents. 2010;20:819–835.
24. Swaminathan S, Khanna N. Experimental dengue vaccines. In: Giese M, editor. Molecular vaccine-from prophylaxis to therapy. Heidelberg: Springer-Verlag; 2013. p. 135–151.
25. Capeding MR, Tran NH, Hadinegoro SRS, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomized, observer-masked, placebo-controlled trial. Lancet. 2014;384:1358–1365.
26. Villar L, Dayan GH, Arredondo-García JL, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. New Eng J Med. 2015;372:113–123.
27. Halamantha B, et al. Investigational drugs in early development for treating dengue infection. Journal Expert Opinion on Investigational drug. Volume 25, 2016- Issue 9.
28. Fang He, et al. Modulation of human humoral immune response through orally administered bovine colostrum. FEMS Immunol Med Microbiol (2001) 31 (2): 93-96.

29. Swey-Shen C., et al. Transforming growth factor B1 is a bifunctional immune regulator for mucosal IgA responses. Cellular Immunology.1990. Volume 128, Issue 2: 353-361
30. Janusz M, et al. Colostral Proline-rich Polypeptides- immunoregulatory properties and Prostpects of Therapeutic Use in Alzheimer's Disease. Current Alzheimer Research, Volume 7, Number 4, June 2010. Pp 323-333 (11).
31. Patiroglu, Turkan. The effect of bovine colostrum on viral upper respiratory tract infections in children with Immunoglobulin A deficiency. 2013. https://www.researchgate.net/publication/305423014_Effects_of_Bovine_Colostrum_on_Recurrent_Respiratory_Tract_Infections_and_Diarrhea_in_children
32. Robert P, Institute of Nutritional Research, Bell Kenz Pharma. Inc. http://igcocolostrum.com, accessed Feb 4, 2017.
33. Oliveria R., et al, Serum cytokine/chemokine profiles in patients with dengue fever (DF) and Dengue hemorrhagic fever (FHD) by using protein array Journal of clinical virology, 02172017
34. Thein T-L, Leo Y-S, Fisher DA, Low JG, Oh HML, Gan VC, et al. (2013) Risk Factors for Fatality among Confirmed Adult Dengue Inpatients in Singapore: A Matched Case-Control Study. *PLoS ONE 8*(11): e81060. doi:10.1371/journal.pone.0081060
35. Velasco, J., Alera, M., Y-pil-Cardenas, C., Dimaano, E., Jarman, R., Chinnawirotpisan, P., Thaisomboonsuk B., Yoon, I., Cummings, D., & Mammen, M. (2014). Demographic Clinical and Laboratory Findings Among Adult and Pediatric Patients Hospitalized with Dengue in the Philippines. *Southest Asian Journal of Tropical Medicine and Public Health,* 45 (2), 337-344.
36. Tahir J, Khalid M, Ghulam M., Nakhshab C. Changing hematologic parameters in dengue viral infection. JAMC Vol 24. No 1 (2012)
37. Butt N, et al. Hematologic and biochemical indicators for early diagnosis of dengue viral infection. J Coll Physicians Pak 2008