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Effectiveness of Steroids in Thrombocytopenia in Dengue Patients – A Review

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ABSTRACT

Dengue is a mosquito-borne viral disease. Dengue hemorrhagic fever (DHF) is a deadly complication of dengue fever (DF) characterized by increased vascular permeability and clotting problems. Dengue shock syndrome (DSS) is another complication of DF that is characterized by circulatory failure and rapid progress to critical state of shock. This article provides an insight into the contributory factors, pathogenesis, and management of dengue with special focus on the role of corticosteroids in the management of the viral illness.

Keywords: Dengue, dengue hemorrhagic fever, dengue shock syndrome, dengue vaccine, corticosteroids

Dengue is a mosquito-borne viral disease, which presents with symptoms of high fever (40^0C), accompanied by severe headache, retro-orbital, muscle and joint pain. Nausea, vomiting, swollen glands and rash are the other typical symptoms. Symptoms last for 2-7 days after the incubation period. In certain cases, complications arise due to plasma leakage, fluid accumulation, respiratory distress and severe bleeding or organ impairment leading to severe dengue.[1] Dengue virus (DENV) are arboviral pathogens belonging to the Flavivirus genus and Flaviviridae family, found mainly in the tropical and subtropical regions. The serotypes include DENV 1 to 4, which are antigenically different and have a single-stranded RNA. The virus transmission occurs via sylvatic, enzootic cycle between mosquitoes and nonhuman primate host, whereas the endemic cycle occurs between humans and mosquitoes.[2] The vector belongs to Aedes family. The primary vector is *Aedes aegypti* and the secondary vector is *Aedes albopictus*.[3,4]

Dengue hemorrhagic fever (DHF) is a deadly complication of dengue fever (DF) characterized by increased vascular permeability and clotting problems. Dengue shock syndrome (DSS) is another complication of DF that is characterized by circulatory failure and rapid progress to critical state of shock. It also presents with petechiae and ecchymoses.[5]

Since it is a viral infection, there is no specific treatment. Treatment of DF mainly involves rest and fluids. Certain observational studies suggest the use of corticosteroids as it seems to benefit in DSS or DHF, preventing the complications of DF. The evidence regarding the beneficial effect of corticosteroids in DF is inconclusive.[6]

HISTORY

The global distribution is about 390 million dengue infections per year, out of which 96 million manifest at any severity. In 2010, it was identified that Asia accounted for 70% of these infections.[7] Mid-1900s saw frequent epidemics of dengue in urban zones and the first outbreak happened in 1963 in Calcutta.[8] In 1943-1944, dengue virus was isolated for the first time and the first virologically proved epidemic of dengue fever occurred in 1963-1964 along the East Coast of India.[9]

VECTOR AND INCUBATION PERIOD

A. aegypti and *A. albopictus* are both the vectors in DENV transmission. The extrinsic incubation period (EIP) is temperature dependent and refers to the time period between viremic blood meal to mosquito becoming infectious. A set of observational studies have set EIP to be 8-12 days. A study conducted by Chan and Johansson identifies EIP to be 5-33 days at 25°C

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and 2-15 days at 30°C.[10] The two significant periods for humans are intrinsic incubation period (IIP) and latent period. IIP basically refers to the onset of symptoms and the latter refers to the time period between infection and onset of infectiousness. According to the World Health Organization (WHO), IIP is about 4-10 days and the Center for Disease Control and Prevention (CDC) mentions it to be around 3-14 days. The Chan and Johansson study identifies it to be 3-10 days.[10]

CONTRIBUTING FACTORS

The increased incidence of dengue in India is due to the unplanned urbanization, changes in environmental factors, host-pathogen interactions and inadequate vector control and population immunologic factors.[8] The environmental factors mainly involve temperature and precipitation. Temperature influences the development rate, mortality and reproduction of mosquitoes, whereas the precipitation influences water habitat for larvae and pupae. Warm temperature and humidity increase the longevity of the adult mosquitoes. It shortens viral incubation period or EIP and facilitates faster viral replication leading to increased transmission.[8]

PATHOGENESIS

The pathogenesis of dengue is unclear, the following is considered as the mechanism involved in dengue infection.

The infection is preceded by the bite of an infected mosquito. This is followed by the dissemination of virus, leading to infection of multiple lymphoid and nonlymphoid tissues. The viremia ensues following the accumulation of virus in the bloodstream leading to the clinical symptoms and interferon expression. Viremia peaks after the onset of fever and then a plateau stage is attained, later gradually declining, depending upon the host immune system.[11] The three phases of dengue include febrile phase, critical phase (plasma leakage) and the recovery phase. DENV binds to the Fcγ receptors on mast cells resulting in cytokine, vascular endothelial growth factors, chymotrypsin production. This leads to endothelial activation and production of inflammatory lipid mediators that increase the vascular permeability and vascular leakage.[12] In the 1960s, Southeast Asia found children dying of a new form of severe dengue named dengue vascular permeability syndrome or DVPS. DVPS is a syndrome that occurs late in the course of an acute dengue illness consisting of thrombocytopenia, altered hemostasis, activated complement, elevated liver transaminase levels with hepatomegaly, vascular permeability and hypoalbuminemia.[13] This condition is identified with second heterotypic DENV infection or infants born to dengue-immune mother, characterized by abrupt onset of fever moving on to cold clammy extremities, slow venous filling, flushed face, restlessness, epigastric pain and petechiae.[14] New evidences point that nonstructural protein 1 (NS1) is a toll-receptor 4 agonist that stimulates primary human myeloid cells to produce cytokines similar to severe dengue disease.[14]

DHF, according to WHO is classified into I, II, III and IV grades, where I and II are mild cases with no shock and III and IV are more severe accompanied by shock. Hemorrhagic manifestation, thrombocytopenia and increased vascular permeability are other signs in DHF, apart from the symptoms seen in DF. Prolonged shock leading to blood being cut-off from gastrointestinal (GI) tract resulting in anoxia, cell death, GI bleed and vasculogenic cytokines are responsible for the hemorrhagic nature of DHF. DENV tropism, activation of complement system, virus virulence, organ pathology, cells of immune system, transient autoimmunity, host genetic factors, antibody-dependent enhancement, cross-reactive T-cell responses, all contribute to DHF.[15]

The defining feature of severe dengue is increased capillary permeability causing plasma leakage, leading to intravascular volume depletion. If the condition is left untreated, it could lead to shock and death.[16]

THROMBOCYTOPENIA AND DENGUE

Thrombocytopenia is one of the most significant clinical manifestations of severe dengue, which can progress to either DHF or DSS. Platelet count decreases due to increased destruction of platelets in peripheral blood or decreased production in bone marrow. The major mechanism of platelet destruction is linked to the activation of complement factor C3 followed by binding of the C5b-9 complex to the platelet surface, which leads to increased platelet destruction.[17]

PREVENTIVE STRATEGIES

Vector control is aimed at the elimination of breeding sites such as water filled man-made containers, improved storage of water and maintenance of environment hygiene.[16] The use of insecticide and larvicide may be effective for a short period of time, but it poses a threat of developing insecticide resistance.[18]

Genetic manipulation of the *A. aegypti* can be a successful tool for pest control. An intracellular bacterium

Wolbachia has been considered as it provides an advantage of invading the population by cytoplasmic incompatibility. A study proved that the Wolbachia infected *A. aegypti* led to decreased pathogen transfer and lifespan of the mosquitoes in Australia.[19,20]

Dengue Vaccine

Vaccination can be considered as an effective mechanism as current methods of preventive strategies for mosquito elimination have become futile.

No antivirals drugs are active against the Flavivirus and control of mosquito vector has thus become difficult. Vaccination remains the most hopeful preventive measure. Inactivated vaccine may be safe but requires booster dose; tetravalent live attenuated vaccine and chimeric vaccine has become the backbone of dengue vaccines.[21] Types of vaccines evaluated include live attenuated, live chimeric, recombinant, inactivated, subunit and DNA vaccine.[22]

The first dengue vaccine is CYD-TDV, which is a live recombinant tetravalent vaccine. It was first registered in Mexico in December 2015, and is given in a 0-6-12 months schedule.[23] Vaccines have been developed correlating to the pathogenesis of dengue. A genetically modified infectious virus clone is an advanced form. Genetic vaccination and use of recombinant virus such as attenuated adenovirus are being investigated.[24] The reactogenicity of vaccine occurs due to the adaptive or acquired immunity.[25] Challenges faced include existence of 4 DENV types, absence of validated animal model, no validated human model, incomplete understanding of the immune profile and poorly immunizing vaccine.[26] A published study shows effective results of CYD-TDV vaccine in children aged 9-16 years.[27] Other candidate vaccines with promising results in phase 1 and 2 trials include a live attenuated tetravalent dengue vaccine, and a recombinant live attenuated tetravalent vaccine.[28-30] Presently, India has two different dengue vaccine candidates, an LAV and a recombinant protein based vaccine DSV4 for testing. But a safe vaccine seems to be out of reach.[31]

MANAGEMENT IN DENGUE

The management of dengue mainly involves fluid replacement and rest. Since it is a viral infection, no antibiotics are effective against it.

Since no suitable antivirals are available, symptomatic treatment is provided in dengue. Antipyretics can be given, the use of nonsteroidal anti-inflammatory agents is to be avoided. Oral hydration is advised to all patients.

Fluid Replacement

Hydration is a necessity in dengue fever. Crystalloids are aqueous solution of mineral salts or other water-soluble molecules, whereas colloids contain large insoluble molecules.

Crystalloids or colloids are considered for fluid replacement therapy. About 0.9% normal saline or Ringer's lactate is the most commonly used crystalloid followed by dextran or gelatin in colloids. In serious circumstances, colloids are used because of their greater osmotic effect and they draw the fluid back to capillary.[32,33]

In cases of DHF I and II, 6 mL/kg/h crystalloid solution for 1-2 hours is given. If the patient shows no improvement, increase IV 10 mL/kg/h for 2 hours along with blood transfusion in case of suspected hemorrhage. In DHF III, IV crystalloid solution of 10-20 mL/kg/h is given; if there is no improvement, consider colloids/crystalloids 10-20 mL/kg over 1 hour along with blood transfusion for suspected hemorrhage. DHF IV is treated with rapid volume replacement of 10-20 mL/kg crystalloid solution as bolus over 15-30 minutes.[34] DHF requires a higher volume of fluid from the 3rd day of fever and from the 5th to 7th day, whereas for DF least amount of fluid is required on the 3rd day.[35]

A study compared two crystalloids, normal saline and lactated Ringer's solution and two colloids, dextran 70 and 3% gelatin, and identified colloid solution to be more beneficial.[36] Another study showed that dextran 70 and 6% hydroxyethyl starch were beneficial but dextran caused more adverse effects. The study also stated that Ringer's lactate is indicated for initial resuscitation in children.[37] Another possible treatment is the use of intravenous immunoglobulin (IVIg) due to its immunomodulatory effect.[38]

Corticosteroids

Corticosteroids have been used in a variety of disease conditions. It is a treatment option widely used.

These are anti-inflammatory agents, synthetic compounds similar to the natural hormones produced by the adrenal glands. In higher doses, they have immunosuppressive properties.[39]

Mechanism of action

The mechanism of action of corticosteroids involves both genomic and nongenomic pathway.

Corticosteroids bind to the glucocorticoid receptors (GR) leading to gene transcription (genomic pathway) or

production of responsive elements (nongenomic) that result in the immunomodulatory effect. Understanding the GR signaling may help in the development of more safe and effective steroid drugs.[40] The pharmacologic action includes cellular functions like development, homeostasis, metabolism, cognition and inflammation.[40,41] They are effective in thrombocytopenia as they inhibit the formation of platelet autoantibodies and reverse the adenosine diphosphate (ADP) activation of platelets leading to decreased destruction of platelets.[42,43]

Platelet count and steroids

The relation between steroids and platelet count has been explored very little. It is believed that steroids can reduce platelet destruction, thereby used in dengue where thrombocytopenia is one of the major complication in dengue.

Corticosteroids are used in immune thrombocytopenic purpura (ITP) as they reduce the rate of platelet destruction and alter the endothelial cell integrity to facilitate primary hemostasis.[44] Prednisone is the conventional treatment in ITP.[45,46] Methylprednisolone[45,47] and dexamethasone[45,48-50] are also used in the treatment, according to randomized trials, as they help in improving the platelet count.[45,51]

Steroids in dengue

Dengue fever has thrombocytopenia as the major complication. Steroids can be used to prevent the platelet destruction. Corticosteroids are used empirically by clinicians as they have the potential for preventing and reducing complications of dengue. The ability of corticosteroids to increase platelet count and reduce its destruction is taken into account. The WHO guidelines for dengue management do not mention corticosteroids. This is due to lack of proper evidence.

The review consists of 13 studies, out of which the majority of participants were children. The earliest evidence came from a small randomized controlled trial, where children with DSS were treated with a tapering dose of hydrocortisone for 3 days. A statistically significant mortality benefit was seen in children 8 years and above.[52] A clinical study showed no benefit with IV hydrocortisone 25 mg/kg/day.[53] This was probably due to the study being unbalanced with 7 in steroid and 19 in control group and single dose of drug.

The studies conducted were divided into three categories, according to the stage of DF when corticosteroid was administered. Table 1 summarizes the studies with the use of steroids in the early stage.

Table 1. Studies with Steroid Use in the Early Stage

Authors/Shock stage of study group	Dose and duration of drug	Results	Explanation for the results
Tam DT, et al /No shock	Low-dose (0.5 mg/kg) or high-dose (2 mg/kg) oral prednisolone for 3 days; within DF for ≤72 hours.	No significant adverse effects or prolongation of viremia. No reduction in the incidence of shock.	High-dose could reduce the risk of shock by up to 43%.
Villar LA, et al/No shock	Methylprednisolone single dose, within 120 hours of fever onset.	Reduces the incidence of bleeding and no prolongation of viremia; no significant adverse events.	Methylprednisolone has the highest receptor affinity among corticosteroids and IV root access is used. Treated early in the course of illness.
Kularatne SA, et al/No shock	IV 4 mg dexamethasone, followed by 2 mg doses 8 hourly for 24 hours.	A low-dose is used; dexamethasone was not effective in achieving a higher rise of platelet count in dengue infection.	Four-day course of high-dose dexamethasone (40 mg/day) is an effective dose. Dexamethasone 10 mg/day was used here.
Shashidhara KC, et al/ No shock	IV dexamethasone 8 mg initially, followed by 4 mg 8 hourly thereafter for 4 days.	A low-dose is used; dexamethasone was not effective in achieving a higher rise of platelet count.	Four-day course of high-dose dexamethasone (40 mg/day) is an effective dose. Dexamethasone 12 mg/day was given here.
Nguyen TH, et al/No shock	Low-dose (0.5 mg/kg) or high-dose (2 mg/kg) oral prednisolone/Fever for <72 hours.	Early prednisolone therapy has little impact on the host immune response or the clinical evolution of dengue.	After corticosteroid administration, it may take longer duration to detect changes of immune markers. In this study, it was checked on Day 1 and Day 2.

The period is between the onset of dengue symptoms to earliest plasma leakage.

The study conducted by Tam et al, had 225 Vietnamese patients with DF for <72 hours, preceding the critical phase. The patients aged between 5 to 20 years were included in the trial. They had three groups - low-dose (0.5 mg/kg), high-dose (2 mg/kg) oral prednisolone and a placebo group. The therapy continued for 3 days and the conclusion obtained was that oral prednisolone during the preliminary phase of DF is not associated with significant adverse events or clinical events nor leads to reduction in the incidence of dengue complications, but it highlighted that high-dose corticosteroids could reduce the risk of shock up to 43%.[54] The study by Nguyen et al had high-dose (2 mg/kg) and low-dose (0.5 mg/kg) of prednisolone administered to patients for 3 days. The concentration of 11 cytokines and chemokines were measured each day for 3 days and it was found that there was no attenuation of acute phase plasma cytokines by prednisolone treatment. The study concluded that there was no reduction in the severity of plasma leakage but there was no prolonged viremia in the prednisolone treated patients.[55] Two other clinical trials saw a rise in the mean platelet count following low- (4 mg initially, followed by 2 mg every 8 h for 24 h) (Kularatne et al) and high- (8 mg initially followed by 4 mg every 8 h for 4 days) doses (Shashidhara et al) of dexamethasone; however, it was ineffective in achieving a higher rise in the platelet count.[56,57]

A positive response to corticosteroid administration in the early phase was obtained from another study conducted by Villar et al that had patients in the age group of 5-15 and more than 15 years. The participants were given a single dose of methylprednisolone and the study concluded that there was a reduction in the incidence of bleeding and ascites.[58]

To summarize the studies, two trials with high and low doses of prednisolone for 3 days and two trials using IV dexamethasone did not show beneficial effects. A study administering a single dose of methylprednisolone showed promising results. No adverse events were reported in all of the trials mentioned above.

Table 2 summarizes the studies with corticosteroid use during intermediate stage.

The phase between the onset of critical stage and before the severe stage of DSS, not including Grade IV of DHF.

The study conducted by Fernando et al, used hydrocortisone 50 mg IV, 4 times a day in DHF I and II patients. Ninety-two percent showed improvement within 72 hours and none of the patients advanced into the leakage phase. Of the participants in the control group treated with standard dengue management protocols, 24% developed complications such as myocarditis,

Table 2. Studies with Corticosteroid Use During the Intermediate Stage

Authors/Shock stage	Dose and duration of drug	Results	Explanation for the results
Fernando S, et al/DHF Grade I and II	IV hydrocortisone 50 mg, 4 times a day for 3 days.	92% improved within 72 hours/24% of controls had myocarditis, hemorrhage, pneumonia.	Pharmacologically effective drug protocol maintained therapeutic drug levels.
Min M, et al/Shock	IV hydrocortisone Day 1: 25 mg/kg, Day 2: 15 mg/kg, Day 3: 10 mg/kg, for 3 days.	A statistically significant mortality benefit was seen.	Pharmacologically effective drug protocol was used.
Sumarmo et al/Shock	A single dose of IV hydrocortisone hemisuccinate, 50 mg/kg.	No value in the treatment.	No sustained effective drug dose was maintained. High-dose effect lasted only for a short period. Hydrocortisone has low receptor affinity than methylprednisolone.
Futrakul P, et al	IV methylprednisolone: 10-30 mg/kg/dose; Single or repeated dose.	9 out of 11 in treatment group survived. All patients in the control group died.	Sustained and effective drug dose was maintained. Single dose may help due to higher receptor affinity of methylprednisolone.
Futrakul P, et al.	IV methylprednisolone: 30 mg/kg. Repeated doses.	Significant hemodynamic improvement.	Sustained and effective drug dose was maintained with using a drug with higher receptor affinity.

hemorrhage and pneumonia.[59,60] A study by Min et al, used tapering dose of IV hydrocortisone: 25 mg/kg on Day 1, 15 mg/kg on Day 2 and 10 mg/kg on Day 3, for 3 days in children 8 years and above. A clinically significant reduction in mortality rate was seen.[52] Two other trials by Futrakul and colleagues in 1981 and 1987 used multiple doses of IV methylprednisolone of 10-30 mg/kg and demonstrated beneficial effects. [61,62]

Another clinical trial conducted with hydrocortisone showed no beneficial effects. A single dose of hydrocortisone 50 mg/kg of body weight was administered to children with DSS. The response to therapy of 47 children in steroid group and that of 50 in control group, was virtually identical and the study concluded that hydrocortisone is of no value in the treatment of DSS.[63]

To be brief, out of the five trials in the intermediate stage, only one which used a single dose of hydrocortisone failed to exhibit beneficial effects. However, the other trials that used multiple doses showed beneficial effects. Table 3 summarizes the studies with corticosteroid use during the late stage.

A study conducted by Tassniyom et al administered a single high-dose of methylprednisolone IV 30 mg/kg or placebo in 63 children with profound dengue shock. Complications such as fever after shock, pneumonia, convulsions, cardiac arrest, pulmonary hemorrhage and positive hemoculture occurrence were not different in treatment and control groups and the study concluded that the drug has no beneficial effect in reducing the mortality in severe DSS.[64] A study by Widya and Martoatmodjo concludes that 30 mg IV hydrocortisone administered every 4-6 hours/day was ineffective; 10 patients died out of 28.[60,65]

Conversely, a study by Premaratna et al showed beneficial effects of corticosteroids. A single dose of IV methylprednisolone 1 g was given to adults in severe DSS stage. The hematological recovery and morbidity after recovery were significantly shorter in the corticosteroid-treated group.[66]

In short, two trials that used single-dose IV methylprednisolone and multiple doses of IV hydrocortisone did not show any beneficial effects, whereas another study using single-dose IV methylprednisolone showed beneficial effects.

From these studies, it can be summarized that IV methylprednisolone in high and multiple doses demonstrated to be the most beneficial. At high doses, steroids intercalate into the cell membrane thereby altering cellular functions and leading to a reduction in calcium and sodium cycling across immune cells. Also, high-dose steroids act in both genomic and nongenomic pathways unlike the low-dose steroids.[67] A single dose of glucocorticoid has short duration of action as the receptor occupation reverts to its original state quickly thereby implying the significance of multiple doses.[68] Methylprednisolone has a quicker penetration of cell membrane and IV shows a rapid peak.[69] High-dose methylprednisolone has more beneficial effects in thrombocytopenia.[47,70,71] The poor response of dexamethasone in trials may be due to inadequate dose and frequency. Even in ITP, a high-dose is preferred.[72]

Table 3. Studies with Corticosteroid Use in Late Stage

Authors/Shock stage	Dose and duration of drug	Results	Explanation for the results
Premaratna R, et al	IV methylprednisolone 1 g single dose.	Hematological recovery, hospital stay, morbidity after recovery were significantly shorter in the corticosteroid groups.	Single high-dose of a drug with higher receptor affinity and low mineralocorticoid action is used. Reduced confounding factors due to better fluid management in 2011 study than 1993 and 1875.
Tassniyom et al/ Profound shock	IV methylprednisolone single dose 30 mg/kg.	Did not reduce mortality in severe DSS, pneumonia, convulsion, cardiac arrest, pulmonary hemorrhage and positive hemoculture.	At profound shock stage of the illness body does not respond to conventional critical care. Fluid management methods might not be better established in 1993 than 2011 that might have masked the benefit effect of corticosteroids.
Widya MS, et al/Most patients profound shock	IV hydrocortisone 30 mg 4-6 hourly (120-180 mg/day).	No effects of corticosteroids in severe DSS.	Hypervolemia due to mineralocorticoid action of hydrocortisone could increase mortality and morbidity at this stage.

It has also been proved that high-dose dexamethasone enables early cessation of steroids without loss of response in ITP and also is used as an initial treatment option.[48,73] Another study reveals that dexamethasone is superior to prednisolone as it has higher response rates, shorter time to response and fewer bleeding events.[72]

A significant observation is that most beneficial effects of corticosteroid administration were found in intermediate stage of DF, shown in studies conducted by Fernando et al, Min et al and Futrakul and colleagues. The reason may be that at this stage the immune-mediated mechanisms, cross-reacting antibodies, cytokines and chemokines are high, which can be suppressed by adequate amount of steroids. Hydrocortisone did not show benefit in the study by Widya and Martoatmodjo possibly due to its mineralocorticoid effect that led to hypervolemia and adverse effects.

Certain studies show that corticosteroids can be beneficial in dengue, but it requires large randomized trials to prove the beneficial effects[74] and other studies show that the beneficial effects of corticosteroids can be obtained if therapeutic blood levels of these drugs are maintained.[60]

CONCLUSION

The relevance of adding steroids to dengue fever treatment is discussed in this review. It has been identified how steroids can reduce complications in dengue such as thrombocytopenia.

To summarize, corticosteroids have beneficial effects in dengue illness, primarily in the intermediate stage where the immunosuppressive mechanism of corticosteroids is put to use. High doses with multiple frequencies of corticosteroids given intravenously have better results. Although methylprednisolone has shown beneficial effects in dengue trials, dexamethasone may be a better agent. It has short onset of response and less bleeding events. Large randomized trials are required to analyze its beneficial properties.

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