

## Dengue: A mosquito borne disease

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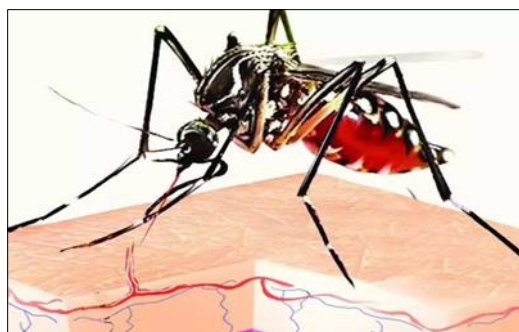
### Abstract

Dengue is a highly infectious disease of the tropical countries. It is caused by any of the 4 serotypes of dengue virus and is transmitted within humans through female *Aedes* mosquitoes. Dengue disease varies from mild fever to severe conditions of dengue hemorrhagic fever and shock syndrome. The optical, electrochemical, microfluidic, enzyme linked immunosorbent assay (ELISA), and smartphone-based biosensors are the main approaches which developed for detection of different biomarkers and serotypes of Dengue virus. Future efforts in miniaturization of these methods open the horizons for development of commercial biosensors for early-diagnosis of Dengue virus infection.

**Keywords:** Dengue; Female *Aedes* Mosquitoes; Dengue virus; ELISA

### 1. Introduction

The dengue virus, a member of the genus *Flavivirus* of the family Flaviviridae, is an arthropod-borne virus that includes four different serotypes (DEN-1, DEN-2, DEN-3, and DEN-4).<sup>1,2</sup>The World Health Organization (WHO) consider dengue as a major global public health challenge in the tropic and subtropic nations.



**Figure 1** Female *Aedes* Mosquito

Dengue has seen a 30-fold upsurge worldwide between 1960 and 2010, due to increased population growth rate, global warming, unplanned urbanization, inefficient mosquito control, frequent air travel, and lack of health care facilities.<sup>3-5</sup> Two and a half billion people reside in dengue-endemic regions<sup>5</sup> and roughly 400 million infections occurring per year, with a mortality rate surpassing 5–20% in some areas.<sup>3-6</sup>

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Dengue infection affects more than 100 countries, including Europe and the United States (USA).<sup>7</sup> The first reported case of dengue like illness in India was in Madras in 1780, the first virologically proved epidemic of DF in India occurred in Calcutta and Eastern Coast of India in 1963-1964.<sup>7,8</sup>

Dengue virus infection presents with a diverse clinical picture that ranges from asymptomatic illness to DF to the severe illness of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS).<sup>4</sup>

Oral mucosal involvement is seen in approximately 30% of patients, although oral features are more frequently associated with DHF than with dengue fever. Dengue virus infection exhibit varied clinical presentation, hence, accurate diagnosis is difficult and relies on laboratory confirmation. The condition is usually self-limiting and antiviral therapy is not currently available. Supportive care with analgesics, hydration with fluid replacement, and sufficient bed rest forms the preferred management strategy.<sup>9</sup>

## 2. History

Dengue virus was isolated in Japan in 1943 by inoculation of serum of patients in suckling mice<sup>10</sup> and at Calcutta (now Kolkata) in 1944 from serum samples of US soldiers<sup>11</sup>. The first epidemic of clinical dengue-like illness was recorded in Madras (now Chennai) in 1780 and the first virologically proved epidemic of DF in India occurred in Calcutta and Eastern Coast of India in 1963-1964<sup>5-7</sup>.

The first major epidemic of the DHF occurred in 1953-1954 in Philippines followed by a quick global spread of epidemics of DF/DHF<sup>13</sup>. DHF was occurring in the adjoining countries but it was absent in India for unknown reasons as all the risk factors were present. The DHF started simmering in various parts of India since 1988<sup>10-12</sup>.

The first major wide spread epidemics of DHF/DSS occurred in India in 1996 involving areas around Delhi<sup>13</sup> and Lucknow<sup>14</sup> and then it spread to all over the country<sup>15</sup>.

## 3. Statistics

In India, dengue cases have seen a steep rise in every second. It has been seen that the number of cases was higher in 2017 (1,88,401) and 2021 (1,93,245) as compare to the dengue cases in 2016(129,166), 2019 (157,315), 2015(99,913), 2018(101,192), and the lowest case in 2020(44,585).<sup>16</sup>

And the death cases in India is more in 2017(325), and 2021(346) as compare to the death cases in 2015(220), 2016(245), 2018(172), 2019(166), and 2020(56).<sup>17</sup>

**Table 1** Statistic of Dengue in India

Sr. No.	Years	No. of cases Found	Death Cases
1.	2015	99.913	220
2.	2016	129.166	245
3.	2017	188.401	325
4.	2018	101.192	172
5.	2019	157.315	166
6.	2020	44.585	56
7.	2021	193.245	346

### 3.1. Further, up to May 31 last year, India recorded just 6,837 dengue cases

This year, the number had already crossed the 10,000-mark by May-end. Every year, an upsurge in cases of dengue was observed between July and November. So far, three dengue deaths have been recorded in India — one each in Puducherry, Uttar Pradesh and Rajasthan. Last year, this number was just two up to May 31. Dengue is a mosquito-borne viral disease that spreads widely in the monsoon season. It is transmitted by the infective bite of the *Aedes Aegypti* mosquito.<sup>18</sup>

## 4. Symptoms

Mild symptoms of dengue can be confused with other illnesses that cause fever, aches and pains, or a rash.<sup>19</sup>

The most common symptom of dengue is fever with any of the following:

- Nausea, vomiting.
- Rash.
- Aches and pains (eye pain, typically behind the eyes, muscle, joint, or bone pain).
- Any warning symptoms.
- Facial flushing.

Symptoms of dengue typically last 2–7 days. Most people will recover after about a week

### 4.1. Severe symptoms<sup>20</sup>

Warning signs of severe dengue is

Watch for signs and symptoms of severe dengue. Warning signs usually begin in the 24–48 hours after your fever has gone away.

Immediately go to a local clinic or emergency room if you or a family member has any of the following symptoms.

- Belly pain, tenderness
- Vomiting (at least 3 times in 24 hours)
- Bleeding from the nose or gum
- Feeling tired, restless, or irritable

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## 5. Diagnosis

Most state health departments and many commercial laboratories perform dengue diagnostic testing.

### 5.1. Clinical management

Dengue virus infection produces a broad spectrum of symptoms, many of which are non-specific. Thus, a diagnosis based only on clinical symptoms is unreliable. Early laboratory confirmation of clinical diagnosis may be valuable because some patients progress over a short period from mild to severe disease and sometimes to death. Early intervention may be life-saving.<sup>21</sup>

Before day 5 of illness, during the febrile period, dengue infections may be diagnosed by virus isolation in cell culture, by detection of viral RNA by nucleic acid amplification tests (NAAT), or by detection of viral antigens by ELISA or rapid tests. Virus isolation in cell culture is usually performed only in laboratories with the necessary infrastructure and technical expertise. For virus culture, it is important to keep blood samples cooled or frozen to preserve the viability of the virus during transport from the patient to the laboratory. The isolation and identification of dengue viruses in cell cultures usually takes several days. Nucleic acid detection assays with excellent performance characteristics may identify dengue viral RNA within 24–48 hours. However, these tests require expensive equipment and reagents and, in order to avoid contamination, tests must observe quality control procedures and must be performed by experienced technicians. NS1 antigen detection kits now becoming commercially available can be used in laboratories with limited equipment and yield results within a few hours. Rapid dengue antigen detection tests can be used in field settings and provide results in less than an hour. Currently, these assays are not type-specific, are expensive and are under evaluation for diagnostic accuracy and cost-effectiveness in multiple settings.

After day 5, dengue viruses and antigens disappear from the blood coincident with the appearance of specific antibodies. NS1 antigen may be detected in some patients for a few days after deference. Dengue serologic tests are more available in dengue-endemic countries than are biological tests. Specimen transport is not a problem as immunoglobulins are stable at tropical room temperatures.<sup>22</sup>

## 5.2. Differential diagnosis

Dengue fever can easily be confused with non-dengue illnesses, particularly in non-epidemic situations. Depending on the geographical origin of the patient, other etiologies – including non-dengue flavivirus infections – should be ruled out.

These include yellow fever, Japanese encephalitis, St Louis encephalitis, Zika, and West Nile, alphaviruses (such as Sinbis and chikungunya), and other causes of fever such as malaria, leptospirosis, typhoid, Rickettsial diseases (*Rickettsia prowazeki*, *R. mooseri*, *R. conori*, *R. rickettsi*, *Orientia tsutsugamushi*, *Coxiella burneti*, etc.), measles, enteroviruses, influenza and influenza-like illnesses, haemorrhagic fevers (Arenaviridae: Junin, etc.; Filoviridae: Marburg, Ebola; Bunyaviridae: hantaviruses, Crimean-Congo haemorrhagic fever, etc.).

Unfortunately, an ideal diagnostic test that permits early and rapid diagnosis, is affordable for different health systems, is easy to perform, and has a robust performance, is not yet available.<sup>23</sup>

## 5.3. Outbreak investigations

During outbreaks some patients may be seen presenting with fever with or without rash during the acute illness stage; some others may present with signs of plasma leakage or shock, and others with signs of hemorrhages, while still others may be observed during the convalescent phase.

One of the priorities in a suspected outbreak is to identify the causative agent so that appropriate public health measures can be taken and physicians can be encouraged to initiate appropriate acute illness management. In such cases, the rapidity and specificity of diagnostic tests is more important than test sensitivity. Samples collected from febrile patients could be tested by nucleic acid methods in a well-equipped laboratory or a broader spectrum of laboratories using an ELISA-based dengue antigen detection kit. If specimens are collected after day 5 of illness, commercial IgM ELISA or sensitive dengue IgM rapid tests may suggest a dengue outbreak, but results are preferably confirmed with reliable serological tests performed in a reference laboratory with broad arbovirus diagnostic capability. Serological assays may be used to determine the extent of outbreaks.<sup>23</sup>

## 5.4. Surveillance

Dengue surveillance systems aim to detect the circulation of specific viruses in the human or mosquito populations. The diagnostic tools used should be sensitive, specific and affordable for the country. Laboratories responsible for surveillance are usually national and/or reference laboratories capable of performing diagnostic tests as described above for dengue and for a broad range of other etiologies.<sup>23</sup>

## 5.5. Vaccine trials

Vaccine trials are performed in order to measure vaccine safety and efficacy in vaccinated persons. The plaque reduction and neutralization test (PRNT) and the microneutralization assays are commonly used to measure protection correlates.<sup>24</sup>

Following primary infections in non-flavivirus immunes, neutralizing antibodies as measured by PRNT may be relatively or completely specific to the infecting virus type<sup>21-22</sup>This assay is the most reliable means of measuring the titre of neutralizing antibodies in the serum of an infected individual as a measure of the level of protection against an infecting virus. The assay is based on the principle that neutralizing antibodies inactivate the virus so that it is no longer able to infect and replicate in target cells.<sup>24</sup>

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## 6. Current dengue diagnostic methods

### 6.1. Virus isolation

Specimens for virus isolation should be collected early in the course of the infection, during the period of viraemia (usually before day 5). Virus may be recovered from serum, plasma and peripheral blood mononuclear cells and attempts may be made from tissues collected at autopsy (e.g., liver, lung, lymph nodes, thymus, bone marrow). Because dengue virus is heat-labile, specimens awaiting transport to the laboratory should be kept in a refrigerator or packed in wet ice. For storage up to 24 hours, specimens should be kept at between +4 °C and +8 °C. For longer storage, specimens should be frozen at -70 °C in a deep-freezer or stored in a liquid nitrogen container. Storage even for short periods at -20 °C is not recommended.

When no other methods are available, clinical specimens may also be inoculated by intracranial route in suckling mice or intrathoracic inoculation of mosquitoes. Newborn animals can develop encephalitis symptoms but with some dengue strains mice may exhibit no signs of illness. Virus antigen is detected in mouse brain or mosquito head squashes by staining with anti-dengue antibodies.<sup>25</sup>

## 6.2. Nucleic acid detection

RNA is heat-labile and therefore specimens for nucleic acid detection must be handled and stored according to the procedures described for virus isolation.<sup>26</sup>

## 6.3. RT-PCR

Since the 1990s, several reverse transcriptase-polymerase chain reaction (RT-PCR) assays have been developed. They offer better sensitivity compared to virus isolation with a much more rapid turnaround time. In situ RT-PCR offers the ability to detect dengue RNA in paraffin-embedded tissues.

All nucleic acid detection assays involve three basic steps: nucleic acid extraction and purification, amplification of the nucleic acid, and detection and characterization of the amplified product. Extraction and purification of viral RNA from the specimen can be done by traditional liquid phase separation methods (e.g., phenol, chloroform) but has been gradually replaced by silica-based commercial kits (beads or columns) that are more reproducible and faster, especially since they can be automated using robotics systems. Many laboratories utilize a nested RT-PCR assay, using universal dengue primers targeting the C/prM region of the genome for an initial reverse transcription and amplification step, followed by a nested PCR amplification that is serotype-specific<sup>26</sup>.

A combination of the four serotype-specific oligonucleotide primers in a single reaction tube (one-step multiplex RT-PCR) is an interesting alternative to the nested RT-PCR. The products of these reactions are separated by electrophoresis on an agarose gel, and the amplification products are visualized as bands of different molecular weights in the agarose gel using ethidium bromide dye, and compared with standard molecular weight markers. In this assay design, dengue serotypes are identified by the size of their bands.<sup>26</sup>

## 6.4. Real-time RT-PCR

The real-time RT-PCR assay is a one-step assay system used to quantitate viral RNA and using primer pairs and probes that are specific to each dengue serotype. The use of a fluorescent probe enables the detection of the reaction products in real time, in a specialized PCR machine, without the need for electrophoresis. Many real-time RT-PCR assays have been developed employing TaqMan or SYBR Green technologies.

The TaqMan real-time PCR is highly specific due to the sequence-specific hybridization of the probe. Nevertheless, primers and probes reported in publications may not be able to detect all dengue virus strains: the sensitivity of the primers and probes depends on their homology with the targeted gene sequence of the particular virus analyzed. The SYBR green real-time RT-PCR has the advantage of simplicity in primer design and uses universal RT-PCR protocols but is theoretically less specific.

Real-time RT-PCR assays are either “single plex” (i.e., detecting only one serotype at a time) or “multiplex” (i.e., able to identify all four serotypes from a single sample). The multiplex assays have the advantage that a single reaction can determine all four serotypes without the potential for introduction of contamination during manipulation of the sample. However, the multiplex real-time RT-PCR assays, although faster, are currently less sensitive than nested RT-PCR assays. An advantage of this method is the ability to determine viral titre in a clinical sample, which may be used to study the pathogenesis of dengue disease<sup>27</sup>.

## 6.5. Isothermal amplification methods

The NASBA (nucleic acid sequence-based amplification) assay is an isothermal RNA-specific amplification assay that does not require thermal cycling instrumentation. The initial stage is a reverse transcription in which the single-stranded RNA target is copied into a double-stranded DNA molecule that serves as a template for RNA transcription. Detection of the amplified RNA is accomplished either by electrochemiluminescence or in real-time with fluorescent-labelled molecular beacon probes.

NASBA has been adapted to dengue virus detection with sensitivity near that of virus isolation in cell cultures and may be a useful method for studying dengue infections in field studies<sup>28</sup>.

Loop mediated amplification methods have also been described but their performance compared to other nucleic acid amplification methods are not known <sup>29</sup>.

## 6.6. Detection of antigens

Until recently, detection of dengue antigens in acute-phase serum was rare in patients with secondary infections because such patients had pre-existing virus-IgG antibody immunocomplexes. New developments in ELISA and dot blot assays directed to the envelop/membrane (E/M) antigen and the non-structural protein 1 (NS1) demonstrated that high concentrations of these antigens in the form of immune complexes could be detected in patients with both primary and secondary dengue infections up to nine days after the onset of illness.

The NS1 glycoprotein is produced by all flaviviruses and is secreted from mammalian cells. NS1 produces a very strong humoral response. Many studies have been directed at using the detection of NS1 to make an early diagnosis of dengue virus infection. Commercial kits for the detection of NS1 antigen are now available, though they do not differentiate between dengue serotypes. Their performance and utility are currently being evaluated by laboratories worldwide, including the WHO/TDR/PDVI laboratory network.

Fluorescent antibody, immune-peroxidase and avidin-biotin enzyme assays allow detection of dengue virus antigen in acetone-fixed leucocytes and in snap-frozen or formalin-fixed tissues collected at autopsy. <sup>30</sup>

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## 7. Serological tests

### 7.1. MAC-ELISA

For the IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) total IgM in patients' sera is captured by anti- $\mu$  chain specific antibodies (specific to human IgM) coated onto a microplate. Dengue-specific antigens, from one to four serotypes (DEN-1, -2, -3, and -4), are bound to the captured anti-dengue IgM antibodies and are detected by monoclonal or polyclonal dengue antibodies directly or indirectly conjugated with an enzyme that will transform a non-colored substrate into colored products. The optical density is measured by spectrophotometer.

Cross-reactivity with other circulating flaviviruses such as Japanese encephalitis, St Louis encephalitis and yellow fever, does not seem to be a problem but some false positives were obtained in sera from patients with malaria, leptospirosis and past dengue infection. These limitations have to be taken into account when using the tests in regions where these pathogens co-circulate. It is recommended that tests be evaluated against a panel of sera from relevant diseases in a particular region before being released to the market. It is not possible to use IgM assays to identify dengue serotypes as these antibodies are broadly cross-reactive even following primary infections. <sup>20</sup>

### 7.2. IgG ELISA

The IgG ELISA is used for the detection of recent or past dengue infections (if paired sera are collected within the correct time frame). This assay uses the same antigens as the MAC-ELISA. The use of E/M-specific capture IgG ELISA (GAC) allows detection of IgG antibodies over a period of 10 months after the infection. IgG antibodies are lifelong as measured by E/M antigen-coated indirect IgG ELISA, but a fourfold or greater increase in IgG antibodies in acute and convalescent paired sera can be used to document recent infections.

Test results correlate well with the hemagglutination-inhibition test. An ELISA inhibition method (EIM) to detect IgG dengue antibodies <sup>31</sup> is also used for the serological diagnosis and surveillance of dengue cases. This system is based in the competition for the antigen sites by IgG dengue antibodies in the sample and the conjugated human IgG anti-dengue.

This method can be used to detect IgG antibodies in serum or plasma and filter-paper stored blood samples and permits identification of a case as a primary or secondary dengue infection <sup>20-22</sup>. In general, IgG ELISA lacks specificity within the flavivirus decomplex groups. Following viral infections, newly produced antibodies are less avid than antibodies produced months or years after infection.

Antibody avidity is used in a few laboratories to discriminate primary and secondary dengue infections. Such tests are not in wide use and are not available commercially. <sup>31</sup>

### 7.3. IgM/IgG ratio

A dengue virus E/M protein-specific IgM/IgG ratio can be used to distinguish primary from secondary dengue virus infections. IgM capture and IgG capture ELISAs are the most common assays for this purpose. In some laboratories, dengue infection is defined as primary if the IgM/IgG OD ratio is greater than 1.2 (using patient's sera at 1/100 dilution) or 1.4 (using patient's sera at 1/20 dilutions). The infection is secondary if the ratio is less than 1.2 or 1.4. This algorithm has also been adopted by some commercial vendors. However, ratios may vary between laboratories, thus indicating the need for better standardization of test performance <sup>32</sup>.

### 7.4. IgA

Positive detection for serum anti-dengue IgA as measured by anti-dengue virus IgA capture ELISA (AAC-ELISA) often occurs one day after that for IgM. The IgA titer peaks around day 8 after onset of fever and decreases rapidly until it is undetectable by day 40. No differences in IgA titers were found by authors between patients with primary or secondary infections. Even though IgA values are generally lower than IgM, both in serum and saliva, the two methods could be performed together to help in interpreting dengue serology. This approach is not used very often and requires additional evaluation. <sup>33</sup>

### 7.5. Hemagglutination-inhibition test

The hemagglutination-inhibition (HI) test is based on the ability of dengue antigens to agglutinate red blood cells (RBC) of ganders or trypsin zed human O RBC. Anti-dengue antibodies in sera can inhibit this agglutination and the potency of this inhibition is measured in an HI test. Serum samples are treated with acetone or kaolin to remove non-specific inhibitors of hemagglutination, and then adsorbed with gander or trypsin zed type O human RBC to remove non-specific agglutinins.

Each batch of antigens and RBC is optimized. PH optima of each dengue haemagglutinin requires the use of multiple different pH buffers for each serotype. Optimally the HI test requires paired sera obtained upon hospital admission (acute) and discharge (convalescent) or paired sera with an interval of more than seven days.

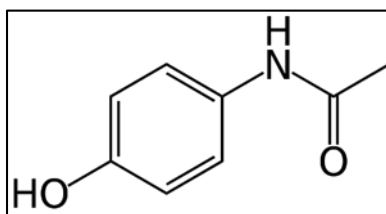
The assay does not discriminate between infections by closely related flaviviruses (e.g., between dengue virus and Japanese encephalitis virus or West Nile virus) nor between immunoglobulin isotypes. The response to a primary infection is characterized by the low level of antibodies in the acute-phase serum drawn before day 5 and a slow elevation of HI antibody titers thereafter.

During secondary dengue infections HI antibody titers rise rapidly, usually exceeding 1:1280. Values below this are generally observed in convalescent sera from patients with primary responses. <sup>34</sup>

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## 8. Treatment

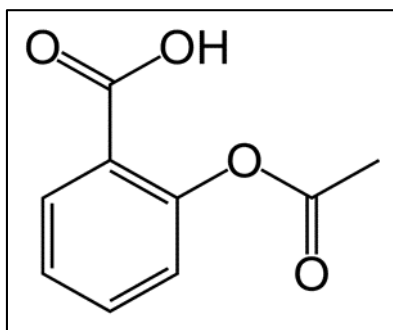
### 8.1. Acetaminophen (paracetamol)



Paracetamol lacks anti-inflammatory action in rheumatic disorders. However, it is less toxic than Aspirin and does not produce anemia and liver damage, which sometimes result from the continued use of acetanilide and acetophenetidine. Paracetamol is used in the treatment of Reduction of fever, Relief of muscle and joint & pain, Relief of cold and flu symptoms, Relief of common headache. <sup>35</sup>

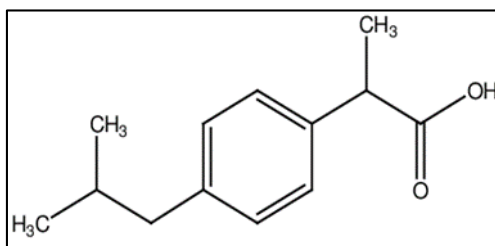
The usual dose for adult is one or two times in 24 hours. Always leave at least 4 hours gap between doses. <sup>35</sup>

## 8.2. Aspirin



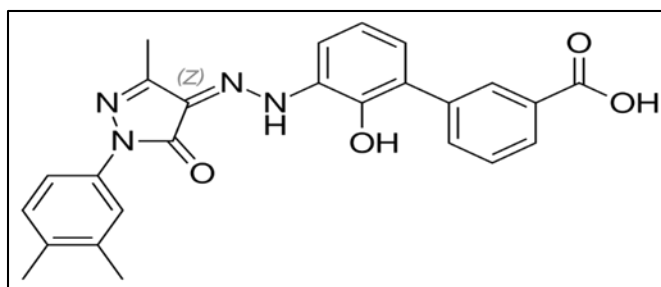
Aspirin is used to reduce fever and to relieve mild to moderate pain from headaches, menstrual periods, arthritis, toothaches, and muscle aches. It works by stopping the production of certain natural substances that causes fever, pain, swelling, and blood clots. The dose in adult is 325-650 mg orally / once every 8 hours. In children under 12 years 10-15 mg/kg orally every 4 hours, up to 60-80 mg/kg/day. In children 12 years and older 325-650 mg orally/rectally once 4-6 hours as needed.<sup>36</sup>

## 8.3. Ibuprofen



Ibuprofen is use to reduce fever and to relieve minor aches and pain from headaches, muscle aches, arthritis, menstrual period, the common cold, toothaches, and backaches. In adults 400 milligrams every 4 hours. In children over 2 years- dose is based on weight and body temperature.<sup>37</sup>

## 8.4. Eltrombopag (Promacta)

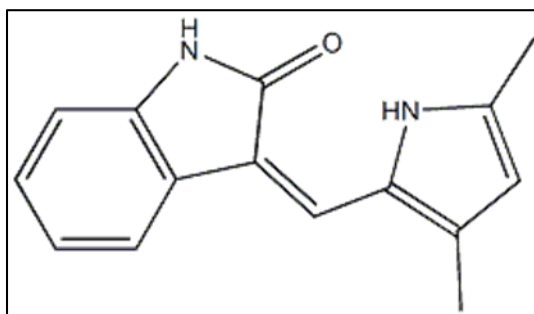


Eltrombopage is used to prevent bleeding episode in adult and children age 1 year and older, who have chronic immune thrombocytopenic purpura (ITP) it is the bleeding conditions caused by a lack of platelets in blood.<sup>38</sup>

In adult and children 12 years of age and older- at first, 150 milligrams once a day for 6 months. In children 2 to 5 years of age- dose is based on weight and must be determined by your doctor.<sup>38</sup>



### 8.5. Romiplostim (Nplate)



Romiplostim is used to increase the number of platelets count in order to decrease the risk of bleeding in adult who have immune thrombocytopenia. Initial dose 1mcg/kg subcutaneous injection once in a week based on acute body weight.<sup>39</sup>

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## 9. Herbal treatment

Various formulations based on plants have also been tried and used by traditional healers and simultaneously been scientifically validated and documented by modern researchers.

### 9.1. *Carica papaya* (*C. papaya*)

Efficacy of *C. papaya* has been reported against the dengue virus. Leaf extract of *C. papaya* was prepared and two spoonful of leaf extract was given to five dengue patients orally, three times per day at an interval of 6 h.

After 24 h of treatment, it was found that there was an increase in the number of platelets. To check the potential of this extract clinical trial was performed. It was randomized, placebo-controlled, double-blind clinical trial and it was executed in 300 patients at 5 different centers.

### 9.2. *Azadirachta indica* (Neem)

As per the in vitro and in vivo study conducted by Parida et al, the leaves extract of *Azadirachta indica* showed positive effect against dengue virus especially for serotype II.<sup>40</sup>

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## 10. Conclusion

Dengue infection is a common mosquito-borne infectious disease that can be seen in many countries around the world. Since it is still an important global public health problem, the issue of management of cases remains an important topic in medicine. This viral infection seems to be difficult to diagnose, since it can be mimicked by other common infections that share the same endemic geography. Although there are many new advanced laboratory investigation tools to help diagnose dengue infection, problems can still be observed. The issue of error in the laboratory diagnosis of dengue has to be mentioned, and the general practitioner has to keep in mind the important points in the diagnosis of dengue. Dengue virus is a currently a problematic global infection. Diagnosis via new molecular-based techniques have become a new hope for early diagnosis, but are still limited due to their costs and standardization. The possibility of treatment of dengue via antiviral drugs is still under investigation.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest:*

Authors declares no conflict of interest.

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