International Journal of Oral Rehabilitation

# Review

# Dengue: Oral manifestations and diagnosis using saliva

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***How to cite:*** *Sitaraman P and Shanmigasundaram K, Dengue: Oral manifestations and diagnosis using saliva. Int J Oral Rehab 2022; Article ID: 20221008; Pages 7.*

***Received : 20.02.2022*** ***Accepted:19.02.2022*** ***Web Published: 31.03.2022***

**Abstract**

Dengue is a vector borne disease and is the most common arbovirus – caused disease in humans. It is known for its increased mortality and morbidity over the years in tropical and subtropical countries of the world. In India, it is an endemic and is a public concern in monsoon due to increased spread of the disease as there is an increase of mosquitoes in the season. It manifests as fever, joint pain and retro orbital pain. Diagnosis is made by clinical and serological examination and frequently involves venepuncture. This review is about early and non – invasive method of diagnosis of dengue using saliva and oral manifestations of dengue.

*Key words*: *Dengue; breakbone fever; dengue hemorrhagic fever; Dengue shock syndrome; manifestations*

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**Introduction:**

Dengue is a mosquito (*Aedes aegypti)* – borne disease caused by a family of RNA viruses called *Flaviviridae.* Five serotypes of the virus have been identified and all of them can cause full spectrum of the disease. Serotypes DENV – 1, DENV –2, DENV – 3, DENV – 4 and DENV – 5 genetically similar to each other. While DENV – 1 to 4 are transmitted to humans, DENV – 5 causes the infection in non – human primates (NHPs).[1] Dengue virus (DENV) causes dengue fever also known as Breakbone Fever or Dandy Fever. The severe forms of the disease are referred to as Dengue Haemorrhagic Fever (DHF) and Dengue shock syndrome (DSS). Dengue has become a notable cause of hospitalization and death among children and adults in tropical countries.[2]

Dengue is seen mostly in urban and semi – urban locations with tropical and subtropical climates. The population at risk is about half the global population. In 2017, over 11000 cases of dengue have been reported when compared to 2016. The cases and the deaths due to dengue fever in India is constantly on the rise since 2010.

The aim of this review is to analyse the various tests employed for diagnosis of dengue using saliva and compile the reported oral manifestations of dengue.

**Transmission of the Virus:**

During feeding of mosquito on humans, the DENV present in the salivary glands of the mosquito enters the dermis of the skin. This results in the infection of immature of Langerhans cells and the keratinocytes in the epidermal layers of the skin. The infected cells migrate to the lymph nodes draining the infected site and further infect the macrophages and monocytes. Consequently, there is amplification of infection and the virus is disseminated through the lymphatic system and subsequently into the systemic circulation.

**Pathogenesis:**

Abnormal hemostasis and plasma leakage are the hall marks of pathophysiology of DHF. Several hypotheses have been put forward to explain the mechanism of interaction between the virus and the immune system of the host.The innate immune mechanism that includes the complement pathway and NK cells as well as the humoral and cell- mediated immune mechanisms are launched in response to the antigenic stimulation.

Dengue infection from one serotype does not elicit long term immunity to the infection caused by other serotypes of dengue virus. It acts a risk factor for severe and fatal dengue.[3] Hence subsequent infection from another viral serotype results in binding of the new virus to the cross reactive non neutralizing antibodies from the previous infection. This facilitates the uptake of the viral antigens into mononuclear phagocytes enabling virus replication. The virus enter the target host cells via clathrin – dependent receptor – mediated endocytosis.[4] Dendritic cell –specific intercellular adhesion molecule – 3 – grabbing non – integrin (DC – SIGN) serves as dengue virus attachment factor on dendritic cells. In secondary infections, the pre – existing antibodies bind to the virions and enable Fcγ receptor – mediated uptake by Fcγ receptor – bearing cells, a process known as antibody – dependent enhancement (ADE). The endocytosis enables the viral antigens to translate in the endoplasmic reticulum followed by replication in the membrane vesicles. The endoplasmic reticulum undergoes rearrangement and expansion during replication of the virus. The virus manipulates the unfolded protein response (UPR) in order to cope up with endoplasmic reticulum stress throughout infection.[5] Non – structural proteins – NS2B, NS3, and NS4A induce UPR to reduce the host cell death during replication.[6] The pathophysiological basis for severe dengue is multi – factorial. The protective and

the pathological outcomes depend on the balance of host immunological and genetic factors and the viral factors.

The possible mechanisms include:[6]

1. Presence of preexisting antibodies that might mediate ADE
2. Infection and activation of innate immune cells
3. Activation of complement system, T and B cells
4. The production of auto - antibodies

**Host Factors:**

Etiological studies have shown that Dengue Hemorrhagic Fever (DHF) is most commonly seen.[7]

1. In infants born to dengue – immune mothers who had primary DENV responses. These infants passively acquired maternal antibodies to primary dengue infection. When infected with secondary dengue, they become more common to DHF.
2. Viral genetics, serotype sequence and time interval between infections can affect the secondary infection outcome
3. Sequence can affect the magnitude of T cell response
4. Increased interval is associated with high risk of disease severity and increased case fatality
5. Other risk factors include bronchial asthma, diabetes and sickle cell anemia.
6. Non – HLA genetic factors like G6PD deficiency, Fcγ receptor IIa, TNF α, IL 10 are also associated with severity of disease.
7. Ethnicity: Black individuals have less severe disease when compared to white individuals.

**Antibody – Dependent Enhancement:**

In dengue mouse model, ADE has shown to be lethal. Virus – antibody complexes bind to Fcγ receptor – bearing cells resulting in increased infected cell – mass and rise in viremia.

**T Cell Activation:**

Secondary infection provokes the activation of serotype cross – reactive memory T lymphocytes with the production of cytokines which ultimately result in plasma leakage. Plasma leakage is a result of the cytokine mediated increase in vascular permeability. The level of T cell activation correlates with disease severity.

**Complement Activation:**

NS1 plays an important role in triggering the activation of complement system. Activated C3a and C5a complexes can be detected in plasma of patients during defervescence and reduction of complement components is patients with DSS.

**Autoimmunity:**

Anti – NS1 antibodies correlate with disease severity and cross – reaction of anti –NS1 antibodies in the liver and endothelial cells and platelets can result in expression of nitric oxide and apoptosis of these cells.

**Clinical Features:**

The course of dengue fever is subdivided into three phases: febrile phase, the critical phase, and the recovery phase.[8] The febrile phase lasts 2 -7 days and the patient experiences sudden onset high grade fever, myalgia, arthralgia, malaise, facial flushing, skin erythema and head ache. It can be difficult to differentiate dengue from other febrile diseases and severe from non – severe dengue cases during acute phase of the disease as the symptoms are non – specific.[9] Therefore careful monitoring of symptoms relating to the progression of the disease is imperative. A decrease in white blood count should serve as an indicator for the high probability of

dengue.The critical phase is marked by increase in capillary permeability and hematocrit and drop in temperature to about 37.5 – 38 °C and remains below this level and lasts for 3 – 7 days. The period of clinically significant plasma leakage usually lasts for 24 – 48 hours. This period is preceded by progressive leukopenia and rapid decrease in platelet count.[10]

**Development of Dengue Shock Syndrome:**

From critical phase, shock develops if critical volume of plasma is lost through leakage. With prolonged shock, there is hypo perfusion to the organs leading to organ impairment, metabolic acidosis and DIC. This in turn leads to severe haemorrhage causing haematocrit to decrease and the white cell count increases in patients with severe bleeding. There is organ impairment even without obvious plasma loss resulting in myocarditis, encephalitis, and hepatitis. Not all patients develop DSS as some improve after the febrile phase of the disease.[10]

**Recovery Phase:**

A gradual reabsorption of the fluid in the extravascular compartment occurs after 48 – 72 hours if the patient survives the first 24 – 48 hours of the critical phase. General well-being of the patient improves, appetite returns, and hemodynamic status is stabilized. The white cell count starts to increase, but platelet count takes a while to recover compared to white cell count. Some patients can develop rash that may be described as "isles of white in the sea of red", pruritus. Bradycardia and changes in electrocardiogram are not uncommon during the recovery stage. Excessive fluid administration during the critical phase and /or the recovery phase can result in massive pleural effusion resulting in respiratory distress and associated with pulmonary oedema or congestive heart failure.[9,10]

**Oral Manifestations of Dengue:**

The most common oral manifestations of are as follows. Few studies reported the following features

- Blisters in different areas of the oral cavity
- Post – extraction bleeding
- Osteonecrosis of jaw
- Thrombocytopenic disorders
- Lingual hematoma

Neurological manifestations relating to dengue like hypoglossal nerve palsy was reported by Jaganathan and Raman, 2014 [11] and persistent metallic taste following dengue was reported by Scully in 2013. [12]According to MS Pedrosa et al [10], identification of oral manifestations can lead to early diagnosis in dengue. Correct medical history regarding the episodes of acute gingival bleeding is imperative in making a correct diagnosis. This makes the dentist to be the first person to accurately diagnose the condition and refer the patient for proper medical management. The dental treatment should be delayed in these patients due to risk of haemorrhagic complications. Prescription of antibiotics and anti – inflammatory drugs should be carefully considered as they can cause hepatic, renal or haematological toxicity.

**Laboratory Diagnosis:**

Laboratory diagnosis of dengue is made by a variety of serological test. The basic serological tests [13] that are performed are hemagglutination – inhibition, complement fixation, neutralization test, immunoglobulin M capture ELISA, and indirect IgG ELISA. These tests are dependent on the increase in the titre of specific antibodies between acute and convalescent phases. Virus isolation and identification is the gold standard for diagnosis of dengue. IgG, IgA, IgM ELISA, real time RT – PCR, NS1 antigen detection, immunofluorescence, paper-based immunoassays, filter paper blood spots, dried blood spots and oral swabs are the tests that are tested using salivary samples and compared to serum/ urine samples to check for the sensitivity and the specificity of the tests in detecting dengue. The timing of sample collection and clinical course of the disease play a major role in accurate diagnosis of dengue. As the serological tests are invasive,

the use of saliva as a diagnostic fluid for dengue has been researched. The collection and use of saliva for the diagnosis of dengue also has other advantages like limited training and no special equipment is needed.[14]

**IgG, IgM, IgA ELISA Tests:**

When DENV - specific antibody ELISA was performed, Andries et al [15], reported that the diagnostic sensitivity of IgG was higher than IgA and IgM ELISA. Similar results were observed in studies reported by Chakravarti et al [14], Spoorthi Ravi Banavar and Vidya G.S.[16], and Andrea J. Cuzzubbo et al.[17] Angel Balmaseda et al [18] reported higher sensitivity of IgM ELISA in saliva when compared to IgA. The low sensitivity of IgA marker could be due to the high concentration of nonspecific IgA present in saliva that can compete with DENV specific IgA. In a study done in 2008 [19] to compare IgM, IgA, and IgG in serum, filter-paper blood spots, and saliva, detection of IgG alone in serum, filter-paper blood spots, or saliva functioned best for measuring DENV infection. Unlike serum and filter-paper blood spots, in primary cases detection of IgM and IgA in saliva was greater than in secondary dengue cases. Grace Yap et al.,[20] found that in the initial phases of the disease, there was a significant increase in the sensitivity of ACA- ELISA in the saliva samples when compared to IgM capture ELISA in sera collected in the first three days and 3-5 days. Increase in the sensitivity of IgM capture ELISA rose after 6 days of fever.

**Real Time RT – PCR:**

Poloni et al [21] reported that virus isolation is possible in salivary samples collected 2 days after the onset of symptoms, and not after 9 days. Previously, in a case report by Y. Mizuno et al [22] stated that virus isolation from saliva was possible by day 7, and in urine samples by days 7, 8 and 14.

**NS1 Antigen Detection:**

Kurhonan et al[23] reported that RNA/ NS1 can be detected in saliva and urine, with a diagnostic sensitivity of 60 % / 56% in saliva. RNA analyses performed on urine, serum and saliva samples collected between 7ys -13 days after the onset of symptoms showed increased sensitivity in urine when compared to serum and saliva. It was also reported that DENV RNA was detectable in saliva samples between 2-6 days after the onset of symptoms. These results are similar to the previous study reported by Hirayama et al. [24]

**Stacking Flow Immunoassays**:

Paper based immunoassays are widely used for home diagnosis. A study based on application of this diagnostic technique to DENV specific IgG was reported by Yi Zhang et al. [25] The study demonstrated successful detection of dengue specific IgG in saliva and classification into primary and secondary dengue. However, the study failed to provide a definitive diagnosis on the current infective status of the patient.

**Management:**

The WHO has formulated complete guidelines for the management of dengue including admission and discharge criteria:[26] Depending on the clinical manifestations and other circumstances, patients may [12] be sent home (Group A), be referred for in-hospital management (Group B), or require emergency treatment and urgent referral (Group C)

**Conclusion:**

Dengue, with its current escalated speed of spread, it is expected to become a pandemic in the near future. With early diagnosis and prompt treatment being the only course of action that helps in curing the disease, the use of saliva as a diagnostic fluid can be utilized as a non – invasive method of diagnosing the disease. Other measures to limit the breeding of the host should be implemented as a precaution. Physicians' should be made well aware of the grouping and the management of the patients so as to prevent the misdiagnosis and treatment that follows the diagnosis.

**Financial support and sponsorship** - Nil

**Conflicts of interest** - There are no conflicts of interest

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