INTRODUCTION

Dengue, the most important mosquito borne viral disease is a leading cause of morbidity and mortality throughout the tropics and subtropics (Gubler, 1998). It has been identified as a clinical entity since 1780 (Rush, 1789). Although, 50 to 100 million dengue infections are known to occur worldwide each year, the true burden of the disease is unknown (Gibbons and Vaughn, 2001). Inspite of several disease surveillance systems in developing countries, the number of reported cases has remarkably increased. Until 1980, the problem of dengue was confined to urban areas only but in recent years due to rapid urbanization the problem has penetrated into rural areas also.

Dengue fever is caused by four different serotypes of dengue virus (DEN 1, DEN 2, DEN 3, and DEN 4) which are closely related antigenically. Infection with one serotype provides life-long immunity to that virus but not to other serotypes. These viruses are the members of the family Flaviviridae. They have a common morphology and genomic structure and all the members share common antigenic determinants. Mature dengue virion consists of 40-50 nm single-stranded non segmented, positive-sense RNA genome surrounded by an icosahedral nucleocapsid of about 30 nm in diameter. This nucleocapsid is covered by a lipid envelope of about 10 nm deep. The complete virion is about 50 nm in diameter. The RNA genome of dengue virus is 10,862 nucleotides long and has an open reading frame of 10,233 nucleotides, which encodes a polypeptide of 3,411 amino acids. The mature virion is made up of single polypeptide which is processed by the viral enzymes and cell proteases to 3 structural proteins (core- proteins C, membrane associated protein - prM and envelope glycoprotein - prE ) and 7
functional proteins viz., NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5). The receptor binding protein of the virus helps the virus to enter the cell and the envelop protein is associated with agglutination of erythrocytes, induction of neutralising antibodies and protective immune response (Noisakran and Pemg, 2008).

Dengue virus is transmitted by female mosquitoes belonging to the genus *Aedes*. The primary and the most important vector is *Aedes aegypti* (Weaver and Barrett, 2004). Other species of mosquitoes such as *Aedes albopictus* and *Aedes polynesiensis* may also act as vectors depending on the geographic location. The principal vector *A. aegypti* is a container breeding, day biting mosquito found in tropical and subtropical areas (Solomon and Mallewa, 2001).

When an infected mosquito bites a person, the virus undergoes multiplication inside the infected person for a period of 4 to 7 days (an average of 3-14 days). The patient may or may not experience symptoms, depending on the virus strain, age, immune status, and the other factors. This is followed by viremia, which is associated with sudden onset of fever and constitutional symptoms lasting for 5-6 days. The dengue virus replicates within the cells of mononuclear phagocyte lineage (macrophages, monocytes and B-cells). Additionally, infection of mast cell, dendritic cells and endothelial cells is known to occur (King et al., 2000). The virus may infect the peripheral blood leukocytes, liver, spleen, lymph node, bone marrow, thymus, heart, kidney, stomach, lungs and possibly the brain suggesting blood brain barrier disruption (Hayes et al., 1992).

Illness caused by one of the four dengue virus serotypes can range from non specific febrile illness to classic dengue fever, which may then progress to severe disease namely Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome
(DSS). Dengue starts with an abrupt onset of high fever (39 - 40 °C) with malaise, headache, nausea, vomiting, myalgia and sometimes abdominal pain. The conjunctivae may be infected and the pharynx may be inflammed. Lymphadenopathy is common. Rash is variable but occurs in 50 % of the patients which lasts from 5 to 7 days (Nimmanitya, 1987). In a small percentage of dengue infections, a more severe form of disease known as DHF occurs. DHF is characterized by acute fever associated with a hemorrhagic manifestation and a tendency to develop shock (DSS), which distinguishes it from DF. A clinical definition of DHF established by the World Health Organization (WHO) is based on the presence of high continuous fever, hemorrhagic manifestations (including a positive tourniquet test), hepatomegaly, thrombocytopenia (Platelet count < 100,000/mm³) and hemoconcentration (hematocrit increased by ≥ 20 % above baseline value). The WHO definition further subdivides DHF into four grades on the basis of the presence of spontaneous bleeding and the presence and severity of shock (WHO, 1986).

In dengue endemic areas, all the three spectrum of dengue infection are common in occurrence. DHF and DSS have typical and stereo typical manifestations characterized by abnormal haemastasis and plasma leakage (Nimmannitya, 1987). These make the clinical diagnosis of DHF and DSS more reliable and accurate (Nimmannitya, 1993). On the other hand, diagnosis of dengue infection at an early stage of illness, before the development of severe manifestation of the disease can be challenging. Unlike DHF, which is solely a clinical diagnosis, the diagnosis of DF relies on the recognition of clinical features along with serologic confirmation or an epidemiological link to the confirmed cases, because the clinical features vary greatly which depend on many factors.
such as the person, the place and the time (Halstead, 1997). More often, they are confused clinically with other infections viz. influenza, measles, typhoid, leptospirosis or any non-specific viral syndrome (George and Lum, 1997). Considering these facts, WHO proposed the clinical diagnosis of DF to “probable DF” and “confirmed DF”. Probable DF is defined by using presumptive support serology, single specimen positive for HI or ELISA and/or with the occurrence of confirmed cases of DF in the same location and time. Confirmed DF needs more specific dengue laboratory support such as virus isolation, four fold rise in the antibody titre and viral antigen demonstration (WHO, 1997).

Early diagnosis of dengue fever though is not often feasible, has critical implication over the individuals and the community. It enables more timely assessment and initiation of supportive clinical management of patients with warning signs of severe disease (Wills et al., 2005). Diagnosis at an early stage, while the patient is still febrile and viremic would additionally help to limit further transmission of virus within households and communities. For these reasons, the need for “clinical and laboratory indicators of early dengue” has been identified as one of the global research priorities by WHO. A systematic review on dengue, identified multiple clinical and laboratory features that could potentially differentiate dengue from other febrile illness. But it was concluded that published studies to date have been hampered by methodological limitations (Potts and Rothman, 2008). Further, clinical features of dengue were noted to vary significantly between age groups and stages of illness (Deparis et al., 1998; Hammond et al., 2005).

Considering the importance of early diagnosis of dengue and the constraints in obtaining more specific laboratory support such as virus isolation, demonstration
of raise in antibody by serology and demonstration of viral antigen, in the present study it was proposed to identify hematological or biochemical markers in serologically confirmed dengue patients which do not require well sophisticated laboratory as early indicators or predictors of dengue infection. One way to identify these parameters is to estimate the alteration in haematological and biochemical parameters and to analyse the data to assess whether the incorporation of these laboratory results in addition to the clinical data could suggest the early clinical and laboratory features predictive of dengue infection in disease endemic areas.

The more common haematological parameters associated with dengue infection are leucopenia, haematocrit, decreased haemoglobin and thrombocytopenia. Leucopenia and thrombocytopenia are felt to relate to bone marrow suppression by dengue virus (La Russa and Innis, 1995). Its use as a diagnostic tool was proposed before the availability of RT-PCR or NS I antigen assay (Pang et al., 1989). It was stated earlier that the presence of leucopenia in older adults that present with an acute febrile illness should trigger a differential diagnosis of dengue for further laboratory confirmation (Low et al., 2011). According to Sawasdivorn et al., (2001), fever in combination with positive Tourniquet test (TT) and leucopenia differentiate dengue fever from other febrile illness. Like leucopenia, laboratory finding of thrombocytopenia also plays a major role in the diagnosis of dengue infection. However, it is a well described feature of DHF rather than DF. Apart from these two major parameters, Erythrocyte Sedimentation Rate (ESR) is also considered as marker of dengue infection. Unaltered ESR is considered as a good indicator of dengue infection. An another haematological parameter namely hemoconcentration is well correlated with DHF
and decrease in haemoglobin level is generally considered as non-specific indicator.

Biochemical markers such as liver enzymes, cholesterol, triglycerides, albumin, minerals and blood urea nitrogen do play a role in the diagnosis of dengue infection (Feingold et al., 1992; Torres et al., 2002; Pachareon et al., 2002; Howarth et al., 2004; Mekmullica et al., 2005; Itoda et al., 2006; Wang et al., 2007; Chhinna et al., 2008; Kumar et al., 2008; Villar-centeno et al., 2008; Wong and Shen 2008; Suvarna and Rane 2009; Jain et al., 2010; Lumpaopong et al., 2010). Hepatic dysfunction is common in dengue infection which is denoted mainly by hepatomegaly and increased serum liver enzymes. The inflammatory process resulting from infection by the dengue virus leads to a parenchymatous lesion which releases aminotransferases such as Aspartate Amino Transferase (AST) and Alanine AminoTransferase (ALT) into the blood. In the acute phase of the disease, an increase occurs in aminotransferases together with other liver enzymes such as Lactate Dehydrogenase (LDH) and Creatinine Kinase (CK). Hence, assessing the level of aminotransferases is useful in the diagnosis of dengue infection (Souza et al., 2007). Moreover, they act as potential markers to differentiate dengue from other febrile illness (OFI) during the early febrile phase of illness (Kalayanarooj et al., 1997). Other parameters such as cholesterol and triglycerides were reported to be altered in the severe forms of dengue (Alvarez et al., 1985; Ray et al., 1999). Hypoalbuminemia due to plasma leakage is again an indicator of the severity of the disease (Villar–Centeno et al., 2008). Alteration in blood urea nitrogen is also correlated well with the severity of the disease.

Keeping the alterations observed in these haematological and biochemical markers during dengue infection in mind, the present study was designed to
evaluate these markers in the early diagnosis of dengue infection. For this purpose, it was planned to include a group of clinically suspected dengue patients along with a comparison group of patients with OFI and also a group of healthy individuals in order to systematically analyse the early features of dengue infection and to differentiate them from OFI. In the absence of an independent gold standard method to assess the accuracy of clinical diagnosis of DF, IgM enzyme linked immunosorbent assay (ELISA) was chosen to confirm the dengue infection by serology in the clinically suspected dengue patients.

The aim of the present study was to identify potential haematological or biochemical markers as predictors of dengue infection at an early stage. To achieve this goal, a two year cross sectional study was conducted in Salem, Tamil Nadu, where dengue cases are reported every year (Unpublished data).
The objectives of the present study are

1. To describe systematically the clinical features of confirmed dengue infection.

2. To identify any clinical features as a marker of early diagnosis of dengue by comparing the clinical features of dengue patients with patients with OFI.

3. To identify specific haematological marker such as platelet count, haemoglobin (Hb), ESR, white blood cell (WBC) and haematocrit as an early indicatory in the diagnosis of dengue by comparing haematological features of dengue patients with OFI patients.

4. To identify specific biochemical markers of dengue such as AST, ALT, ALP, LDH, CPK, Total cholesterol, Low Density Lipoprotein Cholesterol (LDL cholesterol), High Density Lipoprotein Cholesterol (HDL-cholesterol), Very Low Density Lipoprotein Cholesterol (VLDL-cholesterol), Triglycerides (TG), Sodium, Potassium, Calcium, Chloride, Total protein, Albumin, Globulin, Blood Glucose, Conjugated and Unconjugated Bilirubin, Urea, Uric acid, Creatinine and Antioxidants an early indicatory in the diagnosis of dengue by comparing these biochemical features of dengue patients with OFI patients.

5. To determine the difference in the clinical features, haematological and biochemical abnormalities between dengue confirmed children and adults and also between males and females.