Summary
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Epidemic dengue fever (DF) and dengue haemorrhagic fever (DHF) have emerged as a global public health problem in recent decades. The South-East Asia is at the highest risk of DF and DHF, accounting for 52% of the global risk. Dengue outbreaks now occur periodically in India, indicating high morbidity and mortality, as in other high-burden countries. Dengue virus infection (DV) presents with a wide spectrum of clinical presentations from simple febrile illness DF to severe haemorrhage and shock called DHF and DSS. However, DHF and DSS seen in some DV infected patients suggest that host-related factors are involved in the development of DV-induced severe disease. In this study, related genetic, inflammatory and biochemical host risk factors were studied in exploring the immunopathogenesis of severe dengue disease.

Genetic studies were focused on non-HLA genes such as transporters associated with antigen presentation (TAP) and human platelet antigen (HPA) in view of their major role in events leading to the severity of dengue disease. For the first time, TAP gene polymorphisms could influence the selection process that determines which antigen peptides play a role in the pathogenesis of dengue infection. The aim of this study was to investigate the association of TAP gene polymorphism in diverse pathogenesis of dengue infection. Our studies indicated that the heterozygous genotypes at TAP1^133, TAP2^379 and TAP2^665 loci confer susceptibility to DHF. Among the primary infected individuals, homozygous genotypes for ILE at TAP1^133, VAL at TAP2^379 and ASP at TAP1^637 were found to be a protective factor against development of DHF and DSS respectively. This first report on TAP1 and 2 gene polymorphism suggested that heterozygosity at some of the loci studied carried risk for development of severe dengue disease while homozygous patterns at few of these loci were found to protect them.

Recent studies of TAP proteins have provided clear evidence that TAP polymorphism can have pronounced functional effects, which arise during the induction of allo-specific major histocompatibility complex (MHC) class I restricted cytotoxic T-lymphocyte (CTL) responses. Furthermore, mutation analysis of the TAP gene has shown that even a single substitution of an amino acid can result in dramatic change in substrate.
selectivity and hence it has been suggested that TAP genes are potential regulators of the immune response. In this study increased frequency of heterozygosity at TAP gene among DHF might influence the selection of immuno epitopes of dengue virus and hence might lead to vigorous immune responses in them. The outcome of this study will be useful in exploring the influence of host genetic factors in immunopathogenesis of severe dengue disease. Further this data will be of great help in identification of high risk group for DHF and their early management.

Human platelet antigen (HPA) 1 and 2 polymorphic sites on platelet glycoproteins are identified for autoimmune thrombocytopenia and aberrant coagulation profile in certain diseases. In dengue infection, autoimmune destruction of platelets and abnormal coagulation were suggested as one of the phenomena for thrombocytopenia and shock. In the present study we report for the first time that HPA2a/2b and HPA1a/1b genotypes confer susceptibility to secondary DHF and DSS respectively. Homozygous pattern of ‘a’ at HPA2 locus was found to be a protective factor for development of secondary DHF. The evidence points to a significant association of heterozygous genotypes with severe dengue disease than homozygous genotype. Studies on platelet kinetics in patients with DHF have indicated an increase in platelet destruction as a major cause of thrombocytopenia. An investigation reported the presence of an antiplatelet autoantibody in sera from patients infected with dengue virus and thrombocytopenia. Recent reports have found significant association between Platelet-associated IgG and thrombocytopenia in secondary dengue virus infections and presence of both the dengue virus antigen and human immunoglobulins on the platelet surface in patients with dengue hemorrhagic fever. Thrombocytopenia due to increased platelet destruction by an immune mechanism, therefore, may operate among dengue patients, although the precise mechanism(s) for developing thrombocytopenia remain unclear. One of the reasons for thrombocytopenia might be the development of cross reacting autoantibodies against these specific alloantigens on platelets glycoproteins during dengue which might lead to faster clearance of thrombocytes. This data will be of great help in exploring the etiopathogenesis thrombocytopenia in dengue infection.
Production of free radicals is an important immunological defense mechanism operating against pathogenic infections, including viruses. Equilibrium between pro-oxidants and antioxidants in the body is delicately poised balance limiting the possible macromolecule damage caused by free radicals. Disturbance of this equilibrium contributes to a wide array of diseases, as also complications of viral diseases. Pro-inflammatory cytokines secreted during the early phase of viral infection could contribute to oxidative stress, which may contribute to pathogenesis. This study revealed an increase in oxidative stress in dengue viral infection. The thrombocytopenia observed in dengue infection was associated with the extent of lipid peroxidation. The level of oxidative stress was maximal in severe dengue disease and its severity was minimal in DF. During the course of active illness, the severity of oxidative stress increased and the antioxidant status decreased among the DSS and DHF patients. The study indicated also that in dengue infection, plasma proteins undergo increased levels of oxidative changes from early days of illness and these changes can be predictor of imminent severe dengue infection. Further, a significant negative correlation between protein carbonylation and static acid content of plasma proteins suggest that oxidative stress can cause desialylation of plasma glycoproteins. Future studies on endothelium and platelet sialylation status in dengue infection can through more light into the pathogenesis of severe disease. Elevated levels of TNF-α on day 3 suggested that this cytokine could be a potential early clinical marker of the severity of dengue disease. Our study demonstrated a relative predominance in Th1-type reactivity among DF cases in comparison to DHF/DSS. Hence we suggest that an increased Th1 to Th2 shift in dengue-infected patients is associated with severity of the disease. Correlations of lipid peroxidation and TNF-α with hemeconcentration suggest their role in vascular leakage. An association between lipid peroxidation and TNF-α observed might be the preliminary evidence on the interplay of oxidative stress and inflammatory mediators in influencing the immune response in dengue viral infection. The outcome of this study will be useful in explicating the pathogenesis of thrombocytopenia and vascular leakage in severe dengue disease. Protein oxidant markers will be useful in early identification of high risk individuals for severe dengue disease who need early hospitalization. Further, antioxidant treatment can be considered for DHF and DSS as supportive management.