SUMMARY AND CONCLUSIONS
Hodgkin’s disease (HD) is a malignant lymphoma involving primarily the lymphoid tissues. Eventhough remarkable progress has been made in the treatment of HD, knowledge regarding its basic cellular biology is limited. The cell of origin for this malignancy still eludes us, although multiple groups are investigating its etiology. Probably not much work has been done on the etiology of HD in India. Therefore, this study was undertaken.

The study subjects included a total of 82 HD patients consisting of 12 paediatric cases and 70 adult cases, attending the outpatient clinics of Regional Cancer Centre, Trivandrum. For comparison, 85 age and sex matched healthy normal controls were also included. The specific objectives of this study were (1) to determine the genomic stability status of HD patients by employing cytogenetic methods. (2) to assess the status of cellular and humoral immunity in HD patients (3) to elucidate the association between Epstein-Barr Virus (EBV) and Hodgkin’s disease by serological and immunohistochemical studies and (4) to determine whether these aspects could give any clues to the etiopathology of the disease.

Parameters studied were (1) pedigree analysis of HD patients with family history of cancer (2) cytogenetic studies to determine the constitutional chromosome abnormalities if any and mutagen (Bleomycin) induced chromosome sensitivity as measures of genomic instability of HD patients.
(3) enumeration of total and high affinity rosette forming cells (TRFC and HARFC) and estimation of circulating immune complexes to assess the immune status of HD patients

(4) quantitation of immunoglobulin G (IgG) antibody against Epstein Barr viral capsid antigen (EBVCA) in the sera of HD patients

(5) immunohistochemical detection of EBV specific latent membrane protein-1 (LMP-1) in HD tissues. The results were compared with healthy normal controls using appropriate statistical methods.

Out of the 82 HD patients, 18 showed familial aggregation of other cancers. On analysing the pedigrees of HD patients with family history of cancer, other cancers like breast cancer, oral cancer, colon cancer, uterine and cervical cancer, stomach cancer, liver cancer, lung cancer and leukemias were seen among first or second degree relatives. But none of the patients had familial aggregation of HD among relatives. One noticeable feature of these HD patients with family history of other cancers was the occurrence of similar histologic subtype. Majority of the HD patients had nodular sclerosis (12/18). Besides, 6 patients had mixed cellularity HD. Another noticeable feature was the early age of onset of the disease in patients with family history of other cancers. About 67% of these HD patients were under 35 years of age.

Employing peripheral blood lymphocyte microculture methodology, the constitutional chromosome markers, if any, and the mutagen induced sensitivity of the lymphocytic chromosomes of the study subjects were investigated. Majority of the patients (91%) and all the controls showed normal
chromosome constitution. However, constitutional abnormalities (numerical and structural) in the lymphocytic chromosomes were recorded in 7 HD patients (9%). Chromosome numbers 12 and 21 were the most frequently involved chromosomes in patients with constitutional abnormalities.

Compared to controls, HD patients showed significant increase in spontaneous chromosomal aberrations. The increase in spontaneous chromosomal breaks were more pronounced in HD patients with family history of other cancers than the sporadic patients or controls. Thus increased genomic instability was expressed by HD patients with family history of other cancer.

With regard to bleomycin induced chromosome sensitivity, HD patients expressed increased sensitivity values (82%) compared to healthy controls (2%). Compared to sporadic patients, HD patients with family history of other cancers showed increased sensitivity values. Thus mutagen induced chromosome sensitivity was also found to be an indirect indicator of genomic instability. These results indicated that a good correlation exist between mutagen sensitivity and cancer susceptibility. Most of these HD patients may have defective DNA repair capability, which predispose them to develop cancer. So results from the present study indicate that genomic instability plays a significant role in the etiopathogenesis of HD. It seems that genetic susceptibility is a prerequisite, serving to facilitate the action of some external agent, presumably Epstein Barr Virus.
HD is characterised by a persistent defect in cellular immunity. But it is not clear whether cellular and humoral immunity play any role in the etiology of HD. Hence this study aimed to assess the cellular and humoral immune status of these HD patients. The parameters studied were (1) enumeration of total and high affinity rosette forming cells (TRFC and HARFC) and estimation of circulating immune complexes in HD patients. In the current study the total and high affinity rosette forming cells TRFC & HARFC in HD patients were significantly decreased than in controls. On the other hand, circulating immune complex was significantly increased in patients when compared to that in controls. Patients with untreated HD in all stages exhibited an immune defect characterized by markedly reduced cellular and humoral immunity. It is presumed that a defective immune system responsible for, may be the result of the neoplasm. Conversely, an inherent genetically determined defect resulting in defective cellular immunity may be permissive to the development of HD.

Viral infections have been implicated in the pathogenesis of several malignancies. HD has long been suspected to have an infectious precursor and indirect evidence has implicated Epstein Barr Virus (EBV), an ubiquitous herpes virus, as a causal agent. Hence this study also aimed to elucidate the association between EBV and HD. Parameters studied were (1) quantitation of immunoglobulin G (IgG) antibody against EBV viral capsid antigen in the sera of HD patients (2) immunohistochemical detection of EBV specific latent membrane protein-1 (LMP-1) in HD tissues.
Studies on the pathognomonic role of EBV in HD also showed significant association. Eighty patients and 57 controls had IgG antibody in their sera against the Epstein-Barr viral capsid antigen. Compared to controls, HD patients had significantly higher antibody titres against EBVCA (IgG) thereby indicating that HD is clearly influenced by the presence of EBV. The high levels of antibody titres against EBV observed in the present study, indicate active proliferation of EBV, probably as a result of primary infection with EBV and also reactivation of EB viral genome integrated in the patients lymphocytes. Immunohistochemical staining for EBV specific Latent Membrane Protein 1 (LMP-1) was restricted to Reed-Sternberg cells (RS) and their variants in almost all cases. Overall, 58 patients (71%) showed positivity for EBV LMP-1. Based on the age groups, highest percentage of EBV positivity was observed in children (83%) and elderly adults (77%). According to histologic subtype, highest EBV positivity was in lymphocyte depletion (80%) and in mixed cellularity (75%) subtypes. The strong expression of LMP-1 in the HD tissues studied, implicates its importance in the pathogenesis of HD. Hence, the data from serological and immunohistochemical studies suggest that EBV plays a major role in the etiopathogenesis of a significant proportion of HD.

Thus from this study, it can be concluded that the Epstein Bar Virus is not a mere 'silent passenger' but rather points to an etiologic role for EBV in the pathogenesis of a significant proportion of HD cases. A significant proportion of HD patients exhibited genomic instability and defective
immunity also. It is presumed that EBV alone may not be sufficient for the induction of a malignancy, but has to be complemented by genomic instability and an impairment of antiviral immunity.