DISCUSSION
Cytogenetics

Cancer is a complex disease and certain families express an extraordinarily high rate of aggregation of some types of cancers (Li, 1993). The concept of cancer families has been broadened to encompass all familial occurrence of neoplasms of very dissimilar cell type including childhood cancers, carcinomas, sarcomas and almost all tumour types (Fraumeni, 1977). The inherited basis of certain families with high risk of common cancers has been confirmed with the identification of predisposing mutations or localization of susceptibility genes.

A detailed family history of cancer patients was found to be an easily acceptable and very productive method for the recognition of familial cancer aggregation and the identification of hereditary cancer associations. In this study, 22% of the Hodgkin’s disease patients had family history of other cancers like breast cancer, oral cancer, colon cancer, uterine and cervical cancer, stomach cancer, lung cancer, liver cancer and leukemias among first or second degree relatives. But, none of the patients had familial aggregation of Hodgkin’s disease among relatives.

There are many case reports in the literature of multiple occurrence of Hodgkin’s disease in certain families (Kerzin-Storrar et al., 1983; Halazun et al., 1972; Robertson et al., 1987; Lin et al., 1993), which indicates that heredity may also have a role. Pairs of twins concordant for the disease have been reported (Shibuya et al., 1984), and increases
in the risk of Hodgkin’s disease in the families of these twins ranging from threefold (first-degree relatives) to sevenfold (siblings) have been reported (Robertson et al., 1987; Grufferman et al., 1977; Mack et al., 1995). The apparent absence of an increased risk for spouses of patients would suggest either a genetic factor, or that the exposure or susceptibility must occur in childhood or early adolescence (Grufferman et al., 1977; Vianna et al., 1974).

In the present study, 18 HD patients had history of other cancers among family members. There are other reports on occurrence of Hodgkin’s disease on a familial basis in association with other types of tumors (Buehler et al., 1975; Lynch et al., 1976). Shpilberg et al (1995) reported that familial aggregation was consistent with a genetic predisposition to hematologic neoplasms, and hypothesized that a genetic lesion in a pluripotent stem cell accounts for the diversity of hematologic neoplasms in certain families.

On analysing the pedigrees of familial Hodgkin’s disease patients, one noticeable feature was the occurrence of similar histologic subtype. Majority of the familial Hodgkin’s disease patients had nodular sclerosis (12/18 patients). Besides, 6 patients had mixed cellularity Hodgkin’s disease. Mack et al (1995) reported that nodular sclerosing Hodgkin’s disease accounts for a majority of the diagnoses in the pairs of concordant monozygotic twins. A large majority of the 53 reported sibships with multiple cases of Hodgkin’s disease consisted solely of or included multiple cases of nodular sclerosis (Armata et al., 1991; Martin et al., 1985; Gracz et al., 1979). In another study, Haim et al (1982) reported that mixed cellularity was the most common subtype in familial
cases. The results from the present study are in agreement with the above reports.

Another noticeable feature was that early age of onset of the disease in patients with family history of other cancers. About 67% of HD patients with family history of other cancers were under 35 years of age. Early age of onset of cancer is a characteristic feature of familial cancers.

Familial Hodgkin’s disease (FHD) is a well defined entity (Grufferman et al., 1977). As with sporadic Hodgkin’s disease (HD), the causes are still unclear. Most investigations of FHD have explored the association of HLA phenotype and disease susceptibility (Greene et al., 1979). However, it is likely that only a subset of the FHD cases are due to an HLA-linked susceptibility gene (Chakravarthi et al., 1986).

Many reports strongly indicate that genetic susceptibility rather than an environmental factor is responsible for familial clustering of Hodgkin’s disease (Robertson et al., 1987; Mack et al., 1995; Diehl and Tesch, 1995). Little is known about the environmental risk factors for FHD (Greene et al., 1978; Lynch et al., 1992). Mack et al (1995) reported that in a subgroup of patients with Hodgkin’s disease, an inherited genetic defect is the first step in a multistep process of the genesis of lymphomas.
Genomic instability in HD

The loss of stability of the genome is becoming accepted as one of the most important aspects of carcinogenesis, and the numerous genetic changes associated with the cancer cell implicate genomic instability as contributing to the neoplastic phenotype (Morgan et al., 1996). Genomic instability is characterized by the increased rate of acquisition of alterations in the mammalian genome. These changes encompass a diverse set of biological endpoints including karyotypic abnormalities, gene mutation and amplification, cellular transformation, clonal heterogeneity and delayed reproductive cell death. The molecular genetic and cellular events leading to increased genomic destabilization are not clearly known. Multiple genes are required for the accurate transfer of genetic information from one cell to its progeny during each division cycle. Mutations in any of these genomic stability genes may be an early event in tumorigenesis and may generate the multiple mutations observed in tumors (Morgan et al., 1996).

Genomic instability can be detected at the cytogenetic level by either a high rate of spontaneous chromosome aberrations or an increased sensitivity of lymphocytic chromosomes to certain mutagenic agents. Thus it seems that genomic instability may participate in cancer predisposition in addition to other factors and may account for the heterogeneity of the pathologic status for a given genetic constitution. So in this study, the constitutional abnormalities, spontaneous aberrations and the mutagen induced sensitivity of the lymphocytic chromosomes of Hodgkin's disease
patients were investigated as indicators of genomic instability.

**Constitutional chromosome abnormalities**

Investigations in cancer genetics suggest that specific genetic alterations are etiologically related to specific types of cancer and that in a number of such cases, alterations are cytogenetically detectable. Chromosomal abnormalities are a common feature of malignancies. Despite good mechanistic evidence that specific chromosome rearrangements are etiologic factors for certain forms of cancer, a casual relationship between chromosome damage in surrogated tissues and cancer risk remains by and large a theoretical explanation (Sorsa et al., 1992). Individuals with certain chromosomal abnormalities have been reported to be more susceptible to develop specific types of cancers.

In the present study, majority of the patients (91%) and all the controls showed normal chromosome constitution (males 46,XY and females 46,XX). However, constitutional abnormalities (numerical and structural) in the lymphocytic chromosomes were recorded in 7 HD patients (9%). Chromosome number 12 and 21 were the most frequently involved chromosomes in patients with constitutional abnormalities. Similar type of chromosome abnormalities like the few constitutional abnormalities observed in the present study had been reported in the tissues of HD patients (Anastasi et al., 1987).

A wide variety of abnormalities have been reported in HD tissues with few consistent rearrangements found from case
to case and from study to study (Tilly et al., 1991; Ladanyi et al., 1991; Cabanillas et al., 1988). Thangavelu and LeBeau (1989) reported nonrandom involvement of specific chromosomes in numerical and structural abnormalities. Among Hodgkin's disease - associated chromosomal abnormalities, aneuploidy (100%) with hyperdiploidy (70%) had been reported as the most frequent (Anastasi et al., 1987). Chromosomes 1, 2, 3, 5, 9, 12, 15 and 21 are often triplicated. In a few cases, a loss of chromosomes, for example chromosomes 6, 10, 13, 15, 17, 18, 21, 22 had been reported. Rearrangements, especially translocations or deletions, were found in two-thirds of cases, often involving 1p, 1q, 2q, 4q, 6q, 8q, 11p, 11q, 12q, 13p, 14q and Xq (Thangavelu and LeBeau, 1989; Schouten et al., 1989; Tilly et al., 1991, Ankathil et al., 1992). Break-points 11q23, 14q32, 6q, 8q24, 11q13 have frequently been associated with B and T cell lymphomas (Cabanillas et al., 1988). Poppema et al (1992) reported that q32 region of chromosome is frequently involved, but a t(14; 18) is extremely infrequent. Non-random karyotype abnormalities have also been found in Hodgkin's disease-derived cell lines (Fonatsch et al., 1990).

Karyotypes with simple chromosome defects simply mirror the genetic instability of lymphocytic cells, which is a well-known phenomenon in Hodgkin's disease (Barrios et al., 1988). It remains unclear why these simple chromosome defects are sometimes clonal. It is known that in a given case of Hodgkin's disease, the copy numbers of certain chromosomes may vary considerably among different hyperdiploid metaphases. Fluorescence immunophenotyping and interphase cytogenetics
analysis (FICTION) has shown that the variability of numerical chromosome aberrations was a real phenomenon in vivo (Weber Matthiesen et al., 1992; 1993; 1995). Thus, not only lymphocytes, but also HRS cells, are affected by genetic instability. Schouten et al (1989) and Tilly et al (1991) found no correlation between any numerical or structural chromosomal abnormalities and histological subtypes or other clinical characteristics.

**Sporadic chromosome aberrations**

Chromosomal alterations play a specific etiological role in the development and progression of human malignancy (Sandberg, 1980). The spontaneous frequency of chromosomal aberrations (structural and numerical) in newborns is of the order of 6 per 1000. Populations exposed to ionizing radiation or to genotoxic chemicals have increased frequencies of chromosomal aberration in their lymphocytes. Many types of human cancers are associated with specific or non-specific chromosomal aberrations (Yunis, 1983). On a population basis, increased frequencies of aberrations are an indication of exposure or susceptibility, a factor which increases the risk to cancer and genetic ill health (Carrano and Natarajan, 1988).

In this study HD patients showed a mean spontaneous chromatid b/c value of $0.0136 \pm 0.006$ and in controls, the value was $0.0068 \pm 0.007$. Compared to controls, HD patients showed significant increase in spontaneous chromosomal aberrations ($P < 0.001$). The increase in spontaneous chromosomal breaks were more pronounced in HD patients with
family history of other cancers than their sporadic counterpart or controls. Thus increased genomic instability was expressed by HD patients with family history of other cancer. Study results indicate that affected HD patients belonging to cancer families are having increased genomic instability than sporadic cancer patients in the general population.

**Mutagen induced Chromosome sensitivity analysis**

Assays utilizing peripheral blood lymphocytes exposed in vitro to powerful mutagen have been investigated extensively as a biomarker of cancer susceptibility. Genetic variations in carcinogen metabolism may result in the accumulation of increased levels of reactive metabolites and DNA damage, whereas other factors may reduce the fidelity of DNA replication or repair (Wienecke and Spitz, 1994). There is a wide spectrum of DNA repair capability within the general population (Cianciulli et al., 1994). In order for an organism to keep functioning, protecting the integrity of its genetic material is of utmost importance. Accumulation of damage to the DNA can lead to harm including cancer. To help prevent such damage, cells have evolved elaborate machinery for repairing the DNA after it is damaged. Thus DNA repair capability is considered as a biomarker of cancer susceptibility. Hsu et al (1989) developed an assay in which chromosomal breakage induced by in vitro exposure to the radiomimetic drug bleomycin is used as an indirect measure of DNA repair capability and hence cancer predisposition.

Bleomycin (BLM), a mixture of low molecular-weight (1500 daltons) peptides is isolated from the fungus streptomyces
verticullus. The bleomycin mixture contains mostly the 42 peptide which consists of a DNA-binding fragment and an iron-binding portion located at the opposite end of the molecule. The primary action of bleomycin is to produce single-strand and double-strand breaks in DNA (Petering et al., 1990). The sequence of events leading to DNA breakage begins with activation of the Fe2+ bleomycin complex. Fe2+ which is bound intimately to five nitrogen containing groups in the bleomycin molecule, undergoes spontaneous or enzymatic activation, gaining an electron, and binding to oxygen. The activated complex of Fe2+, oxygen and bleomycin, then binds to DNA as a result of intercalation by the bithiazole groups of the drug. Highly toxic oxygen intermediates, such as the superoxide or hydroxyl radicals, are then formed that attack the 4'-H of deoxyribose leading to cleavage of the sugar and release of its attached base, usually thymine, cytosine, or thin propenyl adducts (Kozarich et al., 1989).

Bleomycin was chosen as the test mutagen in the present study since it induces chromosome type aberrations in G0 and G1, both chromosome and chromatid-type aberrations in S and G2 phase. Bleomycin (BLM) is an antibiotic and radiomimetic glycopeptide that is routinely used in cancer chemotherapy as an antineoplastic agent (Umezawa et al., 1967). BLM is thought to exert its genotoxic effects through the induction of oxidative damage to DNA. From several cohort studies and other indirect evidences, bleomycin sensitivity appears to have a hereditary basis. It has been suggested that cancer prone individuals have a deficiency in some step of DNA repair, which may represent a type of genetic predisposition to cancer (Hsu et al., 1991).
A significant defect in DNA repair shall be manifested by an enhanced sensitivity to the lethal effects of DNA damaging agents. This has been clearly demonstrated in the present study on 82 HD patients, using bleomycin as the test mutagen. Majority of the HD patients expressed increased sensitivity to the mutagen bleomycin. Hodgkin’s disease patients had a range of 78 to 185 chromatid breaks per 100 metaphases and there were 67 mutagen hypersensitive patients. Controls had a range of 23 to 121 breaks per 100 metaphase and 2 subjects were hypersensitive. Eighty two percent of HD patients and 2% control were in hypersensitive group with respect to bleomycin induced chromosome sensitivity values. Here, increased number of HD patients in hypersensitive group may be due to the inheritance of certain genetic factors which make them more susceptible to mutagen. Mutagen induced mean break/cell value of HD patients was 1.321 ± 0.268. Controls had a break/cell value of 0.612 ± 0.187. Among patients studied the highest sensitivity value was shown by a patient aged 18 having lymphocyte depletion HD. Compared to controls, HD patients showed significant increase in mutagen induced b/c values (P <0.001). In the case of induced break/cell, familial patients had increased range and hence maximum instability were expressed by HD patients with family history of cancer. The result from the present study are in agreement with the reports of Liang et al (1989); Cloos et al (1994); Parshad et al (1996) and Patel et al (1997), because almost all study subjects who were familial cancer patients expressed high mutagen sensitive values. In HD patients, the bleomycin sensitivity behaved as an independent factor. Factors such as age, stage, histology, sex, habits etc. did not influence chromosome sensitivity profile of study subjects.
Spitz et al (1989) studied bleomycin induced chromosome sensitivity in untreated aerodigestive tract malignancies and reported this method as a good biomarker for exploring the variable host susceptibility to the action of environmental carcinogens. Liang et al (1989) studied a cancer prone family cytogenetically to determine if certain chromosomal abnormalities might have predisposed members to develop diverse types of malignancies. Cloos et al (1994, 1996) utilized bleomycin sensitivity assay to identify head and neck squamous cell carcinoma patients (HNSCC) who are at higher risk for development of multiple primary tumours. Cianciulli et al (1994) reported increased genomic instability in colorectal cancer patients than controls. Ankathil et al (1995, 1996) reported mutagen induced chromosome sensitivity assay as a potential cytogenetic marker for predicting cancer susceptibility in colon and oral cancer families. Jyothish et al (1997) reported that familial colon cancer patients expressed genomic instability compared to sporadic patients and controls. Thus a good correlation seems to exist between an individual's DNA repair capability and cancer susceptibility. The observations from the present study are in agreement with the above reports.

An individual's proneness to develop chromosome breaks reflect two major parameters, the subject's exposure to clastogens and the inherent chromosome instability. In the latter parameter is also included the ability to efficiently repair any damage that might have occurred to the DNA string. Today, patients with hereditary cancer predisposition are the most extensively investigated and the results thus obtained
may help to shed some light on the possible role of risk factors like constitutional genomic instability in sporadically occurring neoplasia also (Cianciulli et al., 1994).

The mutagen hypersensitivity of the HD patients suggests a common genetic determinant that may be an important factor for cancer development. It is likely that the affected individuals have deficiencies in their general cancer protective mechanisms such as those of DNA repair and recombination which are essential for maintaining the integrity of their DNA. Mutagen hypersensitive individuals may be deficient in the repair genes that affect the efficiency of repair of the DNA lesions induced by Bleomycin. So results from the present study indicate that genomic instability plays a significant role in the etiopathogenesis of HD. It is also possible that the predisposition to HD is probably based upon multiple genetic factors. It seems that genetic susceptibility is a prerequisite, serving to facilitate the action of some external agent, presumably Epstein Barr Virus.

**EBV Association**

The unusually heterogeneous clinical, histologic and epidemiologic characteristics of HD have suggested that it is either a single disease entity with a complex host response or 2 or 3 etiologically distinct conditions. For either interpretation, an infectious precursor has long been proposed. This hypothesis was prompted by clinical symptoms of HD such as cyclic fevers and night sweats, by the morphologic appearance of reactive tissue surrounding the malignant (Reed-Sternberg) cells and by epidemiologic findings of a bimodal age-incidence
curve, geographic variation in the incidence in young persons and childhood social-class risk factors consistent with HD as an uncommon outcome of delayed infection in young adults (Kaplan, 1980; MacMahon, 1966; Correa and O’Conor, 1971; Gutensohn and Cole, 1981).

In contrast to most malignancies, Hodgkin’s disease has a bimodal age-incidence curve (Gutensohn and Cole, 1980). In developed countries, the first peak is seen in young adults between the ages of 15 and 34 years, whereas the second peak occurs in the elderly. It has been suggested that the increased incidence of HD in young adults in economically advantaged populations may be related to the effect of delayed age at primary infection with a common virus (Gutensohn and Cole, 1977; 1981). Support for this has come from studies of childhood social environment that have shown that factors favouring late infection with common viruses, such as small sibship size, early birth order, high maternal education, and low-density housing in childhood, are associated with an increased risk of HD in young adults (Gutensohn and Cole, 1980, 1981). The risk of HD in older age groups is independent of social class variables. In economically developing countries, the first peak is less pronounced and occurs in children between the ages of 5 and 15 years, a fall in incidence being seen during young adulthood. In the current study, the disease was observed to be relatively common in young adulthood in Kerala. This is in agreement with the report of Ramadas et al (1994). Here the second peak occurs in older adults.
In India, mixed cellularity has been reported as the most common histologic subtype encountered and nodular sclerosis as the second one (Rama Rao, 1996; Naresh, 1996; Ramadas et al., 1994). In the present study also, mixed cellularity was observed as the most common histologic subtype, which is well in agreement with the above reports.

The incidence of the different histological subtypes of HD also shows variation with age. Thus, the mixed cellularity (MC) subtype of HD has a bimodal curve with a first peak between the ages of 15 and 34 years and a second peak in the older ages (> 50 years). In contrast, the nodular sclerosis (NS) subtype was the predominant type among children. Mixed cellularity (MC) subtype of HD shows an increased incidence with age; if an infectious agent is involved in the pathogenesis of this group of HD, then it is perhaps more likely to be as a result of reactivation of infection following age-related decline in T cell immunity. A possibly important aetiological agent documented in the etiopathogenesis of HD is the Epstein-Barr Virus (EBV).

**EBV and Hodgkin’s Disease**

**A. Epidemiological and Serological evidence of an association between EBV and HD**

1. **Epidemiological evidence**

   Viral infections have been implicated in the pathogenesis of several malignancies. If HD is related to infection with a common virus, then EBV is a leading candidate for such an agent. The epidemiology of HD occurring in young adults is similar to that of infectious mononucleosis.
Furthermore, cohort studies have shown a two to threefold increased risk of developing HD following infectious mononucleosis (Miller and Beebe, 1973; Rosdahl et al., 1974; Munoz et al., 1978). The excess risk of HD is greatest within 3 years of the diagnosis of infectious mononucleosis. Thus, Epstein Barr Virus has been implicated in the pathogenesis of Hodgkin's disease. An association between EBV and HD has been suggested by the findings of increased titres of EBV induced antibodies in the sera of HD patients by an increased risk of getting HD after infections mononucleosis and by epidemiological evidence. Various epidemiological studies have suggested a possible role for EBV in HD (Mueller, 1987b; Glaser et al., 1997).

The Epstein-Barr Virus (EBV) was originally discovered in cell lines derived from African Burkitt's lymphoma by Epstein, Achong and Barr, and was the first virus shown to be associated with a human cancer (Epstein et al., 1964).

EBV has a worldwide distribution; approximately 90% of adults carry the virus. Most infections occur within the first years of life and are asymptomatic (Okano et al., 1988). EBV infects humans via salivary contact in most nosocomial infections (Morgan et al., 1979; Sumaya et al., 1986, Taylor et al., 1994). In affluent populations with high standards of hygiene, primary EBV infections may not occur until late adolescence or adulthood (Henle and Henle, 1973). Occasionally, some infections have also been transmitted by blood transfusion or bonemarrow transplantation (BMT) (Gratama et al., 1991; Gratama and Ernberg, 1995).

EBV has been linked with a growing number of types of human cancer (Rickinson and Kieff, 1996). These include
Burkitt's lymphoma (BL), nasopharyngeal carcinoma (NPC), Hodgkin's disease (HD), peripheral T-cell lymphomas (PTL), immunoblastic lymphomas in the immunosuppressed and some adenocarcinomas of the stomach.

Biologically, EBV is a B-lymphotropic cellular activator that transforms B-lymphocytes into indefinitely propagating lymphocytoid cell lines in vitro (Miller, 1990a). The virus gains entrance into susceptible lymphoid cells via the complement receptor C3d (also known as CR2 or CD21) (Fingeroth et al., 1984). Expression of this 120 kDa glycoprotein is important for infection of T-lymphocytes and epithelial cells (Fischer et al., 1991; Sixbey et al., 1984). This receptor molecule alone does not necessarily lead to infection, since its expression on a variety of cell lines permits viral absorption but not infection (Liebowitz and Kieff, 1993). Thus, it seems likely that the presence of accessory molecules or cellular transcription factors may also be important host determinants.

**EBV genome and gene expression**

Epstein-Barr Virus, a member of the gamma-herpes virus subfamily, is an enveloped icosahedral virus containing a 172,000 base pair double-stranded linear DNA (Baer et al., 1984). Upon infection, the viral DNA is transported into the nucleus, where it exists predominantly as an extrachromosomal circular molecule (episome) (Yates et al., 1985). The EBV episome consists of short and long unique sequences (US and UL, respectively) separated by a large internal repeat sequence (IRI) and flanked by terminal repeats (TR) at either end of
the genome. The long unique region itself is subdivided by several repetitive elements (IR2, IR3 and IR4). The terminal repeats contain variable numbers of identical 500 base pair sequences arranged as tandem repeats (Hudson et al., 1985) which serve as cohesive sites during the circularization process, thus leading to formation of an episome.

The EBV genome contains over 100 open reading frames (ORFs) potentially encoding for as many polypeptides (Baer et al., 1984). However, only a relatively small proportion of these proteins which are expressed either during latent or lytic viral state have been characterized.

In latent infected, EBV-immortalised lymphoblastoid cell lines, nine proteins are regularly expressed: six nuclear antigens (EBNA-1, 2, 3A, 3B, 3C and LP) and three membrane proteins (latent membrane proteins (LMP) - 1, LMP-2A and 2B (Kieff and Liebowitz, 1990).

EBNA-1 is a protein of about 76 kDa. Its size varies among different EBV isolates and is therefore a useful marker for the identification of individual EBV strains (Yates et al., 1984). EBNA-2 is a specific transactivator of viral and cellular genes. Mainly based on differences in the EBNA-2 open reading frame, two types of EBV have been identified (Calender et al., 1990). EBV-1 and EBV-2 (Sixbey et al., 1989; Jilg et al., 1990). Little is known about the function of the EBNA-3 gene family. EBNA-3C is essential for EBV transformation of B lymphocytes (Robertson et al., 1995;). EBNA leader protein (LP) is transcribed from the leader sequences preceding the EBNA mRNA (Mannick et al., 1991).
Latent Membrane Protein - 1 (LMP-1) is the only EBV-encoded protein with a proven transforming activity (Wang et al., 1985). In vitro, LMP-1 is essential for EBV-mediated transformation of B-lymphocytes into lymphocytoid cell lines and induces many of the signs of activation of infected B-cells like expression of CD23, CD11a, CD18, CD58 and intracellular adhesion molecule-1 (ICAM-1) (Wang et al., 1990). LMP-1 has also transforming effects in rodent fibroblast cell lines, impairing the growth-contact inhibiting effects of cell-cell contact, increasing their ability to grow without substrate, and increasing the tumorigenicity of these cells in nude mice (Wang et al., 1985). In human epithelial cell lines expression of LMP-1 prevents differentiation and increases the expression of receptors for epidermal growth factor (Miller et al., 1995). LMP-1 protein is located in the cell membrane and associates with the cytoskeleton component Vimentin (Liebowitz et al., 1987). It has been recently demonstrated that the cytoplasmic tail of LMP-1 interacts with the tumour necrosis associated factor (TRAF-3), thus communicating a growth signal (Mosialos et al., 1995). In B-lymphocytes, LMP-1 activates expression of cellular protein Bcl-2, a protooncogene that enhances cell survival by preventing apoptosis. The function of the newly identified TP or LMP-2 is as yet unknown (Busson et al., 1995).

In addition to these proteins, two small EBV-encoded non-polyadenylated RNAs (EBER1 and EBER2), comprising 165 and 169 bases, respectively, are found at very high copy numbers (Upto $10^6$ EBER molecules) in the nuclei of latent infected cells (Lerner et al., 1981). The function of these
molecules is largely unknown and it has been speculated that EBER transcripts may regulate translation and message splicing (Toczyski et al., 1991).

Based on the variable expression of the latent gene products, three distinct forms of EBV latent gene expression have been reported, designated latency I, II and III (Rowe et al., 1987; 1992; Henderson et al., 1991). In latency I antigen expression is restricted to EBNA-1 (Rowe et al., 1992). In latency II, there is expression of EBNA-1, LMP-1 and 2. This pattern is observed in Hodgkin’s disease (Pallesen et al., 1991a; Deacon et al., 1993). In latency III, all of the EBNAs and all of the LMPs are expressed (Hudson et al., 1985; Sample et al., 1991)

**EBV Antigens**

Entrance of EBV into lytic state with the production of infectious virions is associated with a broad expression of viral proteins which include enzymes involved in nucleic acid metabolism and formation of viral capsid proteins. The EBV-induced early antigens (EA) are synthesized early, before the onset of DNA replication in the virus replication cycle. This group of antigens can be subdivided into the diffuse (D) and restricted (R) components based on the distribution of the antigens and the sensitivity of the patterns of fixation (Henle et al., 1971). The ZEBRA replication activator (BZLF1) is also an early antigen. Expression of the BZLF1 gene product is both necessary and sufficient for disrupting latency, apparently transactivating other viral genes and initiating the viral cycle (Miller, 1990b; Pallesen et al., 1991b; Herbst 1996, Brousset et al., 1993).
The membrane antigens (MA) are responsible for binding the virus to the receptor and for fusion of the virus envelope with the cell membrane. It consists of at least four major EBV-induced glycoproteins: gp 340/300, gp 250/200, gp 85, and gp 78/55 (Miller, 1990a; Moss et al. 1996).

The VCA system was the first EBV antigen system to be described (Henle and Henle, 1966). The antigen is abundantly expressed in cells undergoing productive infection (Hummel and Kieff, 1982). Antibody to VCA appears after infection and persists for life. It has been the major tool in the study of the epidemiology of EBV infections. VCA has polypeptide and glycoprotein components ranging in size from 26 to 200 kDa, with a 143 kDa polypeptide being the major component (Thorley-Lawson et al., 1982). The gene encoding another late capsid peptide of 22 kd has not been identified (Miller, 1990b).

2. Serological Evidence

The association between Epstein-Barr Virus (EBV) and Hodgkin’s disease (HD) was first noted in serologic studies (Johansson et al., 1970; Levine et al., 1970, 1971). Sera from patients presenting with HD show moderately elevated levels of antibodies to EB VCA, to both components (diffuse and restricted) of EA, and (less consistently) to EBNA, in a proportion of cases, compared with controls (Johansson et al., 1970; Levine et al., 1971; Henle and Henle, 1973; Hesse et al., 1977). These findings have been confirmed in a number of subsequent studies. In the present study, a significant proportion of the patients (87%) with HD had an
elevated level of IgG antibody against the viral capsid antigen (VCA). This suggests an initial severe infection or reactivation of the virus which results in higher antibody responses. This observation is consistent with many previous reports based on the analysis of blood samples obtained before and after treatment (Henle and Henle, 1973; Gotlieb-Stematsky et al., 1975; Evans and Gutensohn, 1984; Levine et al., 1994). Furthermore, Mueller et al. (1989) showed that patients with HD have elevated titers to EBV antigens preceding the diagnosis of HD.

In this study, the effect of multiple factors, which included sex, age, symptoms, histologic subtype and clinical stage on IgG antibody levels to EBV VCA were examined. In these HD patients, sex, age and symptoms had no effect on EBV antibody titers but, disease subtype and stage had a significant association. In the healthy subjects, no effect of sex and age on antibody level was found. Johansson et al. (1970) found elevated anti-VCA titers in about one-half of their patients. In addition, they reported a striking correlation between the level of EBV antibodies and the histological subtype. Their cases were classified using the now outdated Jackson and Parker (1944) HD classification into paragranuloma, granuloma, and HD sarcoma. An inverse relationship was shown between the level of anti-EBV titers and the degree of lymphocyte infiltration in the tumors, high levels being seen in HD sarcoma and near normal titers in the lymphocyte-rich paragranuloma group (Johansson et al., 1970). These studies could not be used to show whether the elevated EBV titers in HD indicated a primary etiological role for the
in order to address this question, Mueller et al (1989), studied sera obtained from HD patients prior to the onset of their disease. They found antibody patterns similar to those reported in presentation sera, with elevated anti-EBV titers to VCA (both IgG and IgA), to the diffuse form of EA, and to EBNA. Altered antibody patterns were most apparent in those sera taken from patients 3 years or more before HD diagnosis. Thus, enhanced EBV activity appears to predate the presentation of HD suggesting that dysregulation of the viral-host balance occurs long before the onset of overt disease. According to Mueller et al (1989), the EBV viral titers in pre-diagnosis HD sera indicate a level of viral activity compatible with a relatively severe, delayed primary EBV infection, or a change in host control in older patients due to diminished immune competence. Although these findings imply a primary pathogenetic role for the virus in HD, it remains possible that elevated EBV titers in HD patients are a secondary marker of abnormal immunity (inherited or acquired) that predisposes to both viral reactivation and to the development of HD. Serologically, HD patients with family history of cancer had higher geometric mean antibody titers (GMTs) to the viral capsid antigen. Lin et al (1996) reported familial HD patients had higher geometric mean antibody titers to the viral capsid antigen and early antigen D (EA-D). Patients with advanced HD had higher IgG to VCA as compared to patients with limited disease (Merk, 1993). In the current study also the results were consistent with the earlier reports. Tan and Henle (1972) reported West Malaysian children had exposure
to EBV relatively early in life, 83% having antibodies to VCA in the 1-2 year age group and 90% to 100% in the older children.

B. Latent EBV gene products in Hodgkin’s Disease Tissues

Poppema et al (1985) were the first to report the immunocytological detection of a latent EBV gene product (EBNA-1) in situ in HRS cells in a HD-like lesion. Examination of the lymphnodes revealed a lesion morphologically indistinguishable from HD (MC type), and large EBNA+ cells were detected in lymphnode suspensions. The conclusion of this case report, that HRS cells in (apparent) HD could express EBV latent antigens, was later confirmed using two separate techniques: (i) Wu et al., (1990) used paraffin section ISH to demonstrate abundant EBER-1 RNA transcripts in HRS cells in six out of eight EBV genome-positive HD cases (ii) Pallesen et al (1991a) used monoclonal antibody frozen section immunohistology to identify abundant LMP-1 expression in HRS cells in about one-half of a large series of HD cases.

These studies showed that both viral transcription and translation could occur in EBV-infected HRS cells, supporting the idea that EBV was not present merely as a “Silent passenger” in HD. That the transformation - associated protein LMP-1 was expressed in HRS cells was thought to be of particular relevance to HD oncogenesis because of its well-established oncogenic and transforming properties in vitro in both human and animal cells.
The viral antigen expression (EBNA1 and LMP1) patterns of tumour cells may reflect not only cell-type specific gene expression, but the immune status of the host as well (Pallesen et al., 1991a; Grasser et al., 1994). HD patients frequently suffer from an impaired immune system, although they are often able to mount high anti-LMP1 antibody titers indicating the presence of an intact humoral immune response against this antigen (Chen et al., 1992). Recently Frisan et al. (1995) have isolated cytotoxic T cells from HD tissues and found HLA class I-restricted EBV-specific cytotoxic T cells in all of three EBV-negative HD tissues. EBV specific cytotoxic cells were present in the peripheral blood of one EBV-positive HD patient (Frisan et al., 1995). This indicates that HD patients do not suffer from a generalized deficiency of EBV-specific immunity. These data indicate that in EBV-associated HD cases, tumour-specific factors may elicit a localized suppression of EBV-specific cellular immunity and thus contribute to the pathogenesis of EBV-positive HD.

1. LMP-1 Expression in HRS cells

Recently included microwave pretreatment of paraffin sections in the staining techniques, and the sensitivity of LMP-1 immunodetection on paraffin sections using CS. 1-4 monoclonal antibodies has been greatly improved. In the current study on LMP-1 expression, results showed a very high prevalence of EBV-positivity in 58 of 82 (71%) HD cases. These findings are in confirmity with the reports of other groups (Herbst et al., 1991; Brousset et al., 1993; Murray et al., 1992; Armstrong et al., 1993; Zhou et al., 1993). Haluska et al.,
(1994) and Chang (1993) also reported that EBV infected HRS cells express LMP-1. In Malaysians a strong association (61%) of EBV with HD has been reported (Peh et al., 1997). They also reported that HD in Indians is more often EBV associated (77%) compared to other ethnic groups.

Other groups have detected LMP-1 in HRS cells in HD in lesser frequencies. The HRS cell LMP-1 expression was reported by Herbst et al (1991; 1992) in 18/47 (38%) and 18/46 (39%) HD cases, respectively; in 36/107 cases (34%) by Delsol et al (1992); in 16/39 cases (41%) by Armstrong et al., (1993); in 22/46 cases (48%) by Murray et al (1992); and in 40/84 cases (48%) by Zhou et al., (1993).

An analysis of LMP-1 at the mRNA level has been performed by Deacon et al., (1993) using reverse transcription PCR. The amplification product was identified in 11 of the 12 EBV (HRS cell) positive but in none of the 5 EBV-negative HD biopsies.

In the present study on 82 HD patients, LMP-1 expression was observed in 58 patients (71%). But no significant difference was observed between LMP-1-positive and - negative cases with regard to sex, age, clinical stage, subtype or systemic symptoms. Also LMP-1 expression in HRS cells correlated with an eventual degree of immune dysfunction in the corresponding patients. HD is rarely diagnosed in children younger than 5 years. Primary infection with EBV occurs silently in young children. The fact that children from lower socioeconomic backgrounds acquire EBV at a younger age than do middle class children, is perhaps reflective of more
In this study 70% of these patients were of low socioeconomic group. Chang et al (1993) and Leoncini et al (1996) have suggested an ill-defined, low socio-economic status-related immunosuppression as a cause for a higher EBV association in their HD patients.

C. EBV association with histological subtypes of HD

Numerous published studies have reported variation in the pattern of association of EBV with the histological subtype of Hodgkin's disease (Pallesen et al., 1991a; 1993; Murray et al., 1992; Delsol, 1992; Oudejans et al., 1997). The association of EBV in Hodgkin's disease being subtype dependent was first noted by Pallesen et al (1991a) and later reported by others from their respective population studies. This observation was again reconfirmed by Weinreb et al (1992) based on the study of various populations from different geographical locations. Hence, with the exception of the report from Jarrett et al (1991) indicating the lack of significant association of EBV with subtype, a strong association of EBV with mixed cellularity Hodgkin's disease appears to be consistently uniform all over the world. The association of MC subtype and EBV has been subsequently confirmed in some (Staal et al., 1989; Weiss et al., 1991; Delsol et al., 1992; Murray et al., 1992; Weinreb et al., 1992; Vestlev et al., 1992) but not all (Herbst et al., 1991; Jarrett et al., 1991) studies. Glaser et al (1997) also reported that histologic subtype was the strongest risk factor for HD being EBV positive. In their study EBV gene products were present in approximately 75% of MC tumours.
In the current study EBV encoded LMP-1 was found in 50% of patients with LP, 70% of those with NS, 75% of those with mixed cellularity and 80% of lymphocyte depletion. The results revealed a high prevalence of EBV among lymphocyte depletion and mixed cellularity Hodgkin's disease. A possible explanation for this difference could be a difference in immunocompetence status of the individuals, ie, patients with mixed cellularity and lymphocyte depletion Hodgkin's disease might have more severely impaired cytotoxic T-cell immunity, permitting the growth of LMP-1 positive Hodgkin and Reed-Sternberg cells. In Kerala, HD is relatively common in young adulthood (15-34) like that of western countries. In this age group, mixed cellularity is the predominant histologic subtype. This may be the reason for the highest EBV positivity in this study. The causes suggested for a higher involvement of EBV in HD in developing countries include certain epidemiological features, especially the histological type of HD (Glaser et al., 1997).

D. EBV positivity and sex of HD patients

In most of the studies, the incidence of Hodgkin's disease has a male predominance. This distribution is most marked in patients younger than 10 years (Spitz et al., 1986). In the present study also, the incidence of Hodgkin's disease has a male predominance. This distribution is most marked in patients younger than 14 years. Among males, highest EBV positivity was observed in the > 50 age groups. In the 0-14 years age group, females had 100% positivity. In the 15-34 years (young adults) age group, females had 50% and males had 68% EBV positivity. The age specific protective
effect of female gender is consistent with a role for female reproductive experience in the development of EBV-positive HD. Although there is no direct evidence regarding reproductive risk for EBV-associated malignancies in general or HD in particular, experimental data do support an interaction of pregnancy-mediated immunosuppressive mechanisms (e.g. via glucocorticoids) with expression of EBV gene products, suggesting that such a biologic mechanism is plausible (Sargent, 1993; Glaser et al., 1995; 1997).

E. EBV-positive Hodgkin's Disease in Different Populations

The prevalence of EBV sequences differs not only between developed and developing countries, but also between different developing countries.

1. EBV in Western HD

PCR overestimates the true frequency of HRS cell EBV infection in HD as it detects virus in nonneoplastic lymphocytes. Accurate estimation of the incidence of EBV in HD in Western patients used in situ techniques of virus detections. The results of LMP-1 immunostaining and EBER ISH from several studies suggested that EBV infected HRS cells in approximately 50% of HD cases (Pallesen et al., 1991 a; Herbst et al., 1992; Armstrong et al., 1992; Weinreb et al., 1992; Murray et al., 1992; Lauritzen et al., 1994; Quintanilla-Martinez et al., 1995; Leoncini et al., 1996).

2. EBV in Asian HD

In Asia, EBV is known to be closely associated with nasopharyngeal carcinoma which is particularly prevalent in
the ethnic Chinese in Southern China, Taiwan, Hong Kong, Malaysia and Singapore. In the same ethnic population there is also a higher frequency of upper aerodigestive tract T-cell/NK cell lymphomas with a similarly strong association with EBV (Chan et al., 1994; Prasad and Rampal, 1992; Peh et al, 1995). Other varieties of peripheral T-cell lymphoma have also been reported to be EBV associated. In Peninsular Malaysia, the association of EBV with nasopharyngeal carcinoma is well documented, with the tumour showing a predilection for the ethnic Chinese, followed by ethnic Malay, but uncommon in the Indians (Prasad and Rampal, 1992).

There are limited reports on the relationship of EBV with cases of Asian Hodgkin’s disease (Zhou et al., 1993; Chan et al., 1995; Tomita et al., 1996). Hodgkin’s disease is uncommon in Asia compared with Caucasian populations, accounting for only 8% of all lymphomas in Hong Kong and Taiwan (Ho et al., 1984; Su et al., 1985) and only 6% of all lymphomas in Japan (Kadin et al., 1983). Hodgkin’s disease has been reported in 18-22% of all lymphomas from mainland China (Harrington et al., 1987; Yan et al., 1991). A study from India (Nair and Krishnaswami, 1990) however, observed a higher frequency (30%) of HD, differing from other major published Asian reports.

In order to study the association between EBV and HD in developing country, we examined 82 cases of HD from Kerala, India for expression of LMP-1 using immunohistochemistry. The EBV-positive HRS cells were found in 58/82 cases (71%). This is well in agreement with the results of other studies.
(Zhou et al., 1993). The EBV gene expression in HRS cells varied according to the histologic subtype, being detected in 30/40 MC (75%), 19/27 NS (70%), 5/10 LP (50%) and 4/5 (80%) lymphocyte depletion Hodgkin’s disease. Thus, the overall frequency as well as the subtype distribution (MC>NS) of Indian EBV-positive HD is similar to that found in China and in other developing countries.

3. Association of EBV-Positive HD with age

A potentially important risk factor for the development of an EBV-related tumour is age (Jarrett et al., 1991; Ambinder et al., 1993). It appears that children infected shortly after the maternal neutralizing antibodies have dissipated are much more likely to develop Hodgkin’s disease than children in whom infection occurs much later in life. Why it is so, is not known.

In the current study, EBV positivity were found in all age groups, including infants. Here EBV positivity was higher in children and older adults. These findings indicate that HD in children is at least as strongly linked to EBV as in adults. This variation of EBV positivity with age is consistent with the multiple-etiology hypothesis, which states that the cause of HD differs by age group. In our population most children have their first EBV infection above 5 years. Earlier Naresh (1996) from India reported that 100% of childhood Hodgkin’s disease were positive for EBV. The substantially increased risks of EBV positive NS and MC in children suggest that timing of infection greatly affects the association of EBV with HD tumours. An early age at infection was strongly
predictive of EBV positivity in both these histologic subtypes. Jarrett et al (1991) reported an excess of EBV-positive HD cases in young (less than 15 years) and older (more than 50 years) patients compared with young adults. These results supported the hypothesis that HD is a heterogeneous condition with different etiologies in different age groups. Other groups however, have not found an association between age and the frequency of EBV-positive HD in Caucasians (Libetta et al., 1990; Coates et al., 1991; Vestlev et al., 1992; Weinreb et al., 1992). That the rate of EBV positive HD in children appears to be at least equal to that in adults is by itself of interest. Since a substantial proportion of children remain EBV seronegative up to the age of 15 years in Western countries, Weinreb et al (1992) proposed that the finding of similar rates of EBV-positive HD at all ages suggest that EBV infection of HRS cells is not coincidental, but rather an indication of a specific etiological role for the virus.

Spring et al (1996) reported more than 90% of paediatric HD in Honduras, India and Pakistan is associated with EBV. It is possible that the very early EBV infection occurring in children in developing countries simply predisposes them to HD, presumably in conjunction with other factors. Peh et al (1997) from Malaysia reported a very high prevalence of EBV-positivity in childhood HD cases (93%), irrespective of the subtype of the lesions or the ethnic origin of the patients. This is in concordance with other studies which indicated a very strong association between EBV and childhood HD (100%) even in widely different populations such as Peruvian (Chang et al., 1993), Honduran (Ambinder et al., 1993) Chinese (Zhou et al., 1993; Chan et al., 1995) and the Kenyan (Weinreb
These observations, together with the previous finding of a clonal episomal virus pattern, strongly suggest that EBV may play an important aetiological role in childhood HD in developing countries, regardless of the ethnicity of the populations.

In the current study, the EBV positivity was found to be associated with age. Thus, 10/12 cases (83%) were EBV positive in patients under 14 years of age, compared with 17/22 cases (77%) in patients above 50 years of age. Young adults and patients in 35-49 years age group also showed 64% and 67% EBV positivity, respectively.

Several authors have reported that EBV in Hodgkin’s disease is most strongly associated with very young and very old, in underdeveloped countries (Ambinder et al., 1993; Armstrong et al., 1993; Chang et al., 1993; Khan et al., 1993).

4. Correlation between EBV (HRS cell) Positive HD and Serology

HD patients frequently show abnormal patterns of EBV serology. So it was of interest to compare EBV expression in HRS cells with serology in individual patients.

The EBV serological status of HD patients is infrequently mentioned in the various studies of EBV tissue expression (Delsol et al., 1992; Brousset et al., 1991). Brousset et al (1991) found no correlation between the presence of EBV DNA in HRS cells in HD shown by ISH and patient serology. Similarly, Mueller 1992 found no correlation between EBV antibody titers and expression of EBV genes in a series of HD cases.
from Massachusetts or cases from Danish service (Pallesen et al., 1993). Levine et al (1994) reported that IgG antibody against VCA is not predictive of the presence or absence of EBV in Reed-Sternberg cells in HD. Enbald et al (1996) also found no correlation between EBV serology and occurrence of EBV sequences in tissues from their HD patients. Hadar et al (1995) from Israel reported 90% of the young adult population in their area is EBV seropositive. But, by immunohistochemistry Benharroch et al (1997) reported 30% of EBV sequences in Israel population.

Jox et al (1996) have postulated a hit-and-run mechanism in EBV-associated transformation. According to this pattern, EBV genomes would integrate in chromosomal areas prone to breakage. This could lead to a selective loss of integrated EBV and explain the lack of correlation between EBV seropositivity and tissue EBV positivity. It is possible that this may account for a variable expression in different ethnic groups and in different geographic areas, but this point needs further clarification. In spite of the EBV seropositivity in most patients, other factors (viruses, exposure to wood, hereditary factors) may play a role in the majority of patients.

F. Influence of EBV infection with clinical parameters

Vestlev et al (1992) and Brousset et al (1991) compared LMP-1 expression with clinical parameters such as sex, age, systemic symptoms, clinical stage, mediastinal involvement and ESR. The LMP-1 expression was significantly associated only with the histological subtype (Vestlev et al., 1992).
In the current study, no significant association was found with any of these parameters.

Interestingly, Weinreb et al (1992) found that LMP expression in HRS cells was independently associated, not only with MC subtype morphology but also with clinical stage IV disease.

Application of molecular biological methods has substantiated the previous seroepidemiological identification of EBV as a major candidate etiologic agent in the development of HD. The combined evidence from PCR, EBV DNA and EBER in-situ hybridization, as well as LMP1 immunostaining studies clearly demonstrated that EBV is present in the tumour cell population of up to 50% of HD cases in Western countries (Spring et al., 1996; Herbst, 1996). A high prevalence of EBV was reported from developing countries by immunohistochemical studies. The bulk of viral genomes was found in monoclonal form in most of these cases, thus indicating that the virus had entered the tumour cells prior to their clonal expansion. In the current study, altered EBV serology preceding the onset of the disease places the virus in the appropriate time frame to have a role in the pathogenesis of HD. All four histological types of HD were represented among these EBV-positive cases, though in varying proportions. HD represents a true neoplasm in conjunction with studies demonstrating monoclonal immunoglobulin gene rearrangements in H-RS cells and clonal karyotypic abnormalities. These observations are reinforced by the detection of LMP1, the only EBV gene with established oncogenic potential, in H-RS
cells of EBV-positive HD cases. It is presumed that the integration of Epstein-Barr Viral genome into the host cell genome results in their malignant transformation and immortality. Thus, results from this study strongly suggests that the Epstein-Barr virus is more than a “silent passenger” and rather points to an etiological role for EBV in the pathogenesis of a significant proportion of HD cases. But, however, the mechanism by which EBV contributes to the development of these tumour is not clearly understood. It seems that EBV alone is not sufficient for the induction of a malignancy but has to be complemented by genetic factors and an impairment of antiviral immunity. Therefore, more information, particularly regarding the interaction between EBV gene products with cellular genes and proteins as well as mechanisms of EBV-specific immunity, is required to conclusively assess the significance of the presently available data for the pathogenesis of HD.

Immunology

The comprehensive knowledge relating to the components of both the humoral and cell mediated immune responses may be of immense help in the study of malignancies. In the present study, the role of humoral and cellular immunity in the etiopathogenesis of Hodgkin’s disease was also assessed.

Patients with untreated Hodgkin’s disease in all stages were reported to exhibit reduced cellular immunity with relatively intact humoral immune responses (Twomey and Rice, 1980; Kumar and Penny, 1982; Romagnani et al., 1985; Griesinger
et al., 1990). The cellular immune defect is manifested by impaired reactivity to intradermal antigens, decreased E-rosette formation (Gupta, 1980; Mukhopadyaya et al., 1987; Fisher, 1982) diminished T-lymphocyte chemotaxis and reduced T-lymphocyte proliferation in response to mitogens or mixed lymphocyte culture (Bjorkholm et al., 1982; Kumar and Penny, 1982; Schulof et al., 1981). The cellular immune defect has been reported to be the result of enhanced sensitivity to suppressor monocytes and T-suppressor cells, in addition to abnormal interleukin-2 production (Slivnick et al., 1989; 1990). Levy et al. (1984) reported depressed natural killer (NK) cell cytotoxicity in patients with untreated Hodgkin’s disease. Patients with advanced disease (stages III or IV or the presence of B symptoms) have been reported to have an inherent T-lymphocyte defect (Eltringham and Kaplan, 1973; Van Rijswijk et al., 1986).

Many studies have demonstrated decreased cell mediated immunity in cancer patients (Scully, 1982; Good 1972; Rajendran et al., 1986; Pillai et al., 1988). It has been generally accepted that lymphocytes particularly T-cells are primarily responsible for cell-mediated immune responses.

The ‘T’ cells may exert critical regulatory controls on the immune responses of ‘B’ lymphocytes. Much effort has been given to evaluate the cellular immune responses of cancer patients by enumerating the rosette forming cells (RFC) (West et al., 1976). The rosette formation assay is considered to be a simple but potentially useful method for diagnosis and monitoring cancer patients, especially those undergoing immunosuppressive and immunostimulatory therapy.
Different population of lymphocytes are involved in antibody and cell mediated immune responses (Hayward and Greaves, 1977).

Washed, unsensitized sheep erythrocytes (SRBC) bind spontaneously to viable, metabolically active human T cells in vitro, a phenomenon referred to as sheep erythrocyte (E) rosette formation (E-RFC) (West et al., 1977). This phenomenon is mediated by the CD2 molecule, a 50 kD Single chain monomorphic glycoprotein expressed on most T cells. Though monoclonal antibodies may be more useful to characterise the different subsets of T lymphocytes, the E-rosette assay seems to be useful as a diagnostic tool, which most reproducibly discriminate normal individuals from cancer patients. The value of rosette forming cells varies from laboratory to laboratory (Jondal et al., 1972; Wybran et al., 1972). The E-rosette formation by human 'T' cells can be influenced by many factors. Even the age of SRBC was reported be an important variable (Vijayakumar and Vasudevan, 1985).

E-rosette forming cells in human peripheral blood can be subdivided into two fractions on the basis of their relative affinity for sheep red blood cells. 'Low affinity' E-RFC and 'high affinity' E-RFC. Later, Weese et al (1980) employed a modified method by which two types of RFCs could be identified and differentiated. The total rosette forming cells (TRFCs) included all T cells which form rosettes with SRBC at low temperature on prolonged incubation whereas the high affinity rosette forming cells (HARFCs) form rosettes at 29°C with fewer number of SRBC. A depression in 'T' cells in the
Peripheral blood of cancer patients were reported as early as in 1973 by Wybran and Fudenberg. West et al. (1976) reported a decrease in 'T' cells in cancer patients but the depression was higher in patients with metastatic cancer. In the present study on HD patients, the TRFC value was found to be decreased. The percentage of TRFC decreased progressively with the progression of the disease (P < 0.001).

Djeu et al. (1977) first reported a depression in RFC at 29°C (which was later termed as the HARFC) in cancer patients. This was later supported by Bashford and Gough (1983). In the current study, majority of the normal control subjects had HARFC values higher than 47% which is in agreement with previous reports (Weese et al., 1980; Bashford and Gough 1983; Vijayakumar et al., 1985). A progressive decrease in the percentage of HARFC with the progression of the clinical stages was also observed in the present study. These results from the present study are in agreement with the previous reports. The changes in TRFC and HARFC values were found to correlate well with the clinical stages and histologic subtypes of HD patients. No significant difference was observed between patients and controls with regard to different age group. A depression in cell mediated immunity characterised by decrease in TRFC and HARFC levels was observed in these HD patients. Fisher and Young (1978) reported that there is a decrease in general immunity in cancer patients, especially in lymphomas. Susan et al. (1980) reported a significant decrease in cell mediated immunity as observed by the reduction in 'T' cells (E-rosettes)
Humoral immunity, which is relatively normal in untreated patients, is depressed in patients following treatment for Hodgkin's disease (Minor et al., 1979; Weitzman et al., 1977). Antibody-dependent cell-mediated cytotoxicity (ADCC) has been reported to be normal in untreated patients with Hodgkin's disease (Weitzman et al., 1977).

Tumour associated antigens may be released from a developing tumour into the extra cellular environment and subsequently be found in free form and/or as circulating immune complexes (CIC) in serum and other body fluids (Price and Baldwin, 1977). Soluble tumour antigens are believed to create a protection against the attack of specific antibodies and lymphocytes by blocking antigen receptors (Bellido et al., 1981). A variety of methods has been developed for the detection and quantitation of circulating immune complexes in the sera of patients with malignant and other diseases (Baldwin et al., 1979; Celeda et al., 1982; Baseler et al., 1984). The method employing precipitation with Poly Ethylene Glycol 6000 (PEG) has been found to be easy and reliable and hence this method was employed in the present study.

The detection and clinical significance of circulating immune complexes in human neoplasia were reported by many workers (Salinas and Wee, 1982; Salinas et al., 1983; Vijayakumar et al., 1986; Remani et al., 1988; Abraham and Balaram, 1987). In several malignancies elevated levels of circulating immune complexes (CIC) have been reported (Theofilopoulous and Dixon, 1979; Rossen et al., 1977). Many workers have reported elevated levels of circulating immune
complexes in breast, cervix and oral cancers (Scully 1982; Vijayakumar et al., 1986; Remani et al., 1988; Abraham and Balaram, 1987). Elevated levels of circulating immune complexes have been reported in lymphomas and leukaemias (Baldwin et al., 1979; Heier et al., 1979; Euler et al., 1983). In the present investigation, elevated levels of circulating immune complexes were detected in the sera of HD patients. Moreover, the levels of CIC were found to be increasing with progression of clinical stages. These results are in agreement with previous reports. Some reports point towards a correlation between levels of immune complexes, tumour burden and prognosis (Hoffken et al., 1978; Carpentier et al., 1977; Amlot et al., 1978; Gupta et al., 1979). Another report indicate no such correlation between the two (Herberman et al., 1981). The observation of increased levels of CIC in advanced stages of HD patients in the present study is contradictory to the report of Nerurkar et al., 1993). They reported elevated levels of CIC only in stage I and stage II of HD patients. Heier et al (1979) reported that the frequency of circulating immune complexes in the sera of HD patients was somewhat higher among patients with advanced disease than among those with localized disease.

Amlot et al (1976) reported increased quantities of immune complexes in the plasma of untreated HD patients. It was suggested that the detection of circulating complexes may help in assessing the severity of HD. The presence of complexes reflects the activity of HD itself. They reported a correlation between CIC levels and general symptoms such as fever, night sweats and weight loss in Hodgkin’s disease.
Based on these observations, it was suggested that CIC induced some of these symptoms. A fall in CIC levels following treatment and remission has been reported in Hodgkin's disease by Brown et al. (1978). Tumour-specific antigen/antibody complexes appear at the time of tumour spread and the breakdown of cell-mediated defence and such a pattern would accord with the picture presented above (Sjogren et al., 1972). According to Scully (1982) the alterations in humoral immunity in cancer patients may be a reflection of altered cell mediated immunity.

Even though the nature of immune complexes is quite unknown, Heimer et al. (1976) showed that besides immunoglobulins, nonimmunoglobulin components were present in the complexes. It has been suggested that immune complexes are capable of blocking cell mediated immune responses (Hellstrom et al., 1977). In the present investigation, a depression in humoral immunity characterised by increase in circulating immune complexes was observed in HD patients. Overproduction of CIC might be due to the constant production of antibody with the continuous supply of antigens by the disease.

There is no clear understanding of the host’s response leading to elevated levels of CIC in the sera of HD patients. The elevated level in CIC may be attributable to the changes in the complement fixing and non-complement fixing of tumour specific antibodies.
Thus the results from this study clearly indicated a defective immune system in HD patients. But these results did not give any conclusive evidence on the role of immune system in the etiopathology of HD. The persistent cellular immune alterations characteristic of HD 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. This hypothesis becomes more important as other data from this study implicate the possible role of Epstein Barr virus in the initiation of the malignant process.