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# Review: Genetic Alterations of Hodgkine's Lymphoma

**Abdul Hussein Moyet AlFaisal [1] , Israa Hussein Hamzah[2], Hayder Isam Taha[1]**

[1]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies / University of Baghdad / Baghdad / Iraq.
[2] Al-Mustansiriayah University / College of science / Baghdad / Iraq.

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**Abstract:** Lymphoma refers to cancer that developed in the lymphatic system. This type of tumor cause swelling in the lymph nodes and other parts of body. There are 35 different type of lymphoma recognized by WHO,five of them are belong to Hodgkin's lymphoma(HL) and other subtypes belong to non Hodgkin lymphoma. Approximately 85% of lymphoma incidences are non-Hodgkin's disease and 15% are Hodgkin lymphoma. Early epidemiologic data suggested that HL develops among persons with a delayed exposure to a ubiquitous infectious agent, Epstein-Barr virus (EBV). A sound of biological basis association has been established between the genetic regions and risk of HL including some chromosomes and genes such as chromosomes 2, 6, 8, and 10 and *REL and GATA3* genes. In HL there are no known specific simple genetic aberrations that would appear to be necessary for the malignant transformation. Alterations of chromosomes 2p, 3q, 6q, 7q, 9p, 13p, 14p and 17q are found more frequently than expected. Several breakpoints are detected non-randomly in HL, including 3q27, 6q15, 7q22, 11q23, 14q32, but translocation partners have not yet been identified. FISH studies performed with these samples led to the identification of gene amplifications, i.e. amplifications of the JAK2 gene on 9p23–p24 and the MDM2 gene on 12q14.

**Keywords:** Hodgkin's lymphoma, Genetic alterations, Breakpoints, Chromosome aberration.

**Corresponding author:** (Email: alfais2000@yahoo.com).

## Introduction

Cancer is one of the main causes of death worldwide (1). Today is considered the second cause of death after cardiovascular diseases, in the world (2). In 2008, it was reported that 12.7 million people were newly diagnosed with cancer and that 7.6 million people died of cancer (3) but According to the latest report by the World Health Organization (WHO), 14.1 million new cancer cases were diagnosed in 2012. That refers to the ratio increased to 11% in comparison with the previous reported of 12.7 million for 2008 during the same period the number of death from cancer increased from 7.6 million to 8.2 million and expected to reach to 19.1 million in 2025 and may be reach as high as 61 percent in the world by 2050 (4).

## Lymphoma

Lymphoma refers to a general name of cancers that develop in the lymphatic system specifically of lymphocyte cells that may be T or B cell and their precursor cells which undergone different malignant changes such as multiply without any proper order tumors which are collections of cancer cells. This type of tumor cause swelling in the lymph nodes and other parts of body (5). Lymphoma is the sixth most

common type of cancer in United States (6,7).

There are 35 different type of lymphoma recognized by the world health organization's classification system for lymphoma. Five of them are subtypes belong to a group of diseases called Hodgkin lymphoma but all other subtypes belong to diseases called non Hodgkin lymphoma. Approximately 85% of lymphoma incidence are non-Hodgkin's disease and 15% are Hodgkin lymphoma (7-11). Hodgkin's lymphoma is a disease in which malignant (cancer) cells form and develops in the lymph system, part of the body's immune system. The lymph system is made up of lymph, lymph vessels, lymph nodes, spleen, thymus, tonsils and bone marrow(12). Because lymph tissue is found throughout the body, Hodgkin lymphoma can begin in almost any part of the body and spread to almost any tissue or organ in the body (13). Lymphomas are divided into two general types: Hodgkin's lymphoma and non Hodgkin's lymphoma. Hodgkin's lymphoma can occur in both adults and children, in pregnant women and non pregnant women and may also occur in patients who have acquired immunodeficiency syndrome (AIDS) (14).

## Cause and Risk Factor

There are no guidelines for preventing Hodgkin's lymphoma; the cause is unknown or multifactorial **(15).** A risk factor is something that statistically increases one's chance of contracting a disease or condition. Risk factors for Hodgkin's lymphoma include Sex, Ages, Family history, History of infectious mononucleosis or infection with Epstein–Barr virus, a causative agent of mononucleosis, Weakened immune system, including infection with HIV or the presence of AIDS, prolonged use of human growth hormone, exposure to exotoxins, such as agent Orange)(14).

## Classification

### RAEL/ WHO classification of HL(16)

In the recent years the classification system of HD has been changed because the Rye system was united or incorporated into the Revised European American Lymphoma (REAL) classification system and reach to international consensus to classify HD into two types depending on the detection of HRS cells or lymphocytic and histolytic (L&H) cells, respectively which are:

**A. Classical Hodgkin's lymphoma (CHL), that has different types are (17):**
   1) Nodular sclerosis Hodgkin's lymphoma.
   2) Lymphocyte - rich classical Hodgkin's lymphoma.
   3) Mixed cellularity Hodgkin's lymphoma.
   4) Lymphocyte–depleted Hodgkin's lymphoma.

**B. Nodular lymphocyte predominant Hodgkin's lymphoma (LPHL).**

**C. Non Hodgkin's lymphoma**

Most Hodgkin's lymphomas are the classical type. The classical type is broken down into the following four subtypes (17):

1. **Nodular sclerosing Hodgkin's lymphoma:** Is the most common subtype and is composed of large tumor*nodules* showing scattered lacunar classical RS cells set in a background of reactive lymphocytes, eosinophils and plasma cells with

varying degrees of collagen fibrosis/*sclerosis*.

2. **Mixed cellularity Hodgkin**'s **lymphoma:** Is a common subtype and is composed of numerous classic RS cells admixed with numerous inflammatory cells including lymphocytes, histiocytes, eosinophils, and plasma cells without sclerosis. This type is most often associated with EBV infection and may be confused with the early, so-called 'cellular' phase of nodular sclerosing CHL.
3. **Lymphocyte depletion Hodgkin**'s **lymphoma:** Is a rare subtype, composed of large numbers of often pleomorphic RS cells with only few reactive lymphocytes which may easily be confused with diffuse large cell lymphoma. Many cases previously classified within this category would now be reclassified under anaplastic large cell lymphoma.
4. **Lymphocyte-rich classical Hodgkin**'s **lymphoma:** Is a rare subtype, show many features which may cause diagnostic confusion with nodular lymphocyte predominant B-cell Non-Hodgkin's Lymphoma (B-NHL). This form also has the most favorable prognosis.

Nodular lymphocyte predominant Hodgkin's lymphoma expresses CD20, and is not currently considered a form of classical Hodgkin's (18). For the other forms, although the traditional B cell markers (such as CD20) are not expressed on all cells, Reed–Sternberg cells are usually of B cell origin (19) (Figure-1). Although Hodgkin's is now frequently grouped with other B cell malignancies, some T cell markers (such as CD2 and CD4) are occasionally expressed. However, this may be an artifact of the ambiguity inherent in the diagnosis. Hodgkin's cells produce interleukin-21 (IL-21), which was once thought to be exclusive to T cells. This feature may explain the behavior of classical Hodgkin's lymphoma, including clusters of other immune cells gathered around HL cells (infiltrate) in cultures(20).

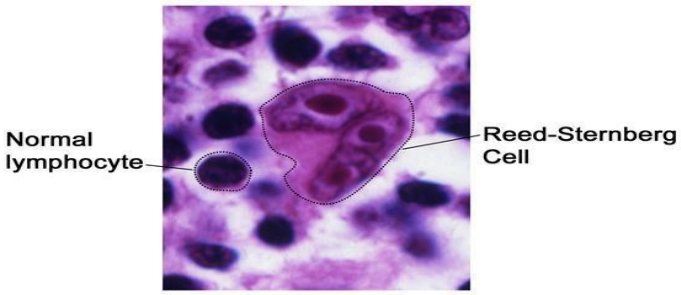


**Figure (1): Reed-Sternberg cell. Reed-Sternberg cells are large, abnormal lymphocytes that may contain more than one nucleus. These cells are found in Hodgkin lymphoma (21).**

## Non Hodgkin lymphoma

This term refers to type of blood cancers called lymphoma infect lymphocytes (white blood cells) which are part of immune system (23). Non Hodgkin lymphomas are much more common than Hodgkins lymphoma (another type of lymphoma) about 80% of all lymphomas diagnosed are non-Hodgkin lymphoma, some of them grow quickly but some other grow slowly (22)(Figure-2).

In the UK, more than 12,000 cases are diagnosed each year and approximately 70,800 new cases and 18,900 death cases from non-Hodgkin lymphoma in the United States. However it has different types but most widespread types of non-Hodgkin's lymphoma include diffuse large B-cell lymphoma and follicular lymphoma (23).

It can occur in lymph node and other organs that contain lymph tissue and it can occur at any age but the chance of developing the condition increase in the older, most cases diagnosed in people over 65. Slightly more men than women are affected which often marked by lymph nodes that are larger than normal, fever, and weight loss(24).

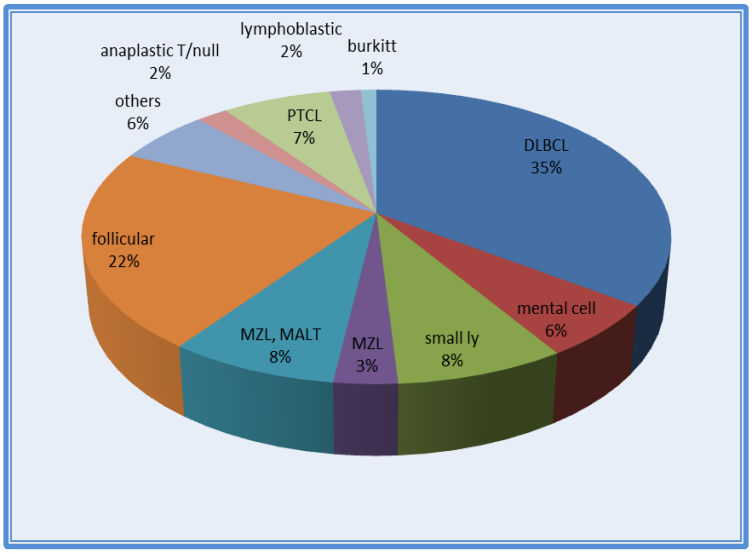


**Figure (2): Non-Hodgkin lymphoma classification.**

**RAEL / WHO classification of NHL(10):**

**B-cell neoplasm's which include:**

a) Precursor B- cell neoplasm's
b) Precursor B lymphoblastic leukemia/ lymphoma
c) Mature B cell neoplasm's that have different types which are :
   1. Chronic lymphocytic leukemia/ small lymphocytic lymphoma.
   2. B cell prolymphomcytic leukemia.
   3. Lymphoplasmacytic leukemia.
   4. Extraosseouplasmactoma.
   5. Splenic marginal zone lymphoma.
   6. Hairy cell leukemia.
   7. Plasma cell myeloma.
   8. Solitary plasmacytoma of bone.
   9. Extra nodal marginal zone Bcell lymphoma of mucosa – associated lymphoid tissue (MALT- lymphoma).
   10. Nodal marginal zone B cell lymphoma.
   11. Follicular lymphoma.
   12. Mantle cell lymphoma.
   13. Diffuse large B cell lymphoma.
   14. Mediatinal (thymic) large B cell lymphoma.
   15. Intravascular large Bcell lymphoma.
   16. Primary effusion lymphoma.
   17. Burkitt lymphoma.

## T- cell and NK cell neoplasm

Precursor T cell neoplasm's which include:

a) Precursor T lymphoblastic leukemia / lymphoma.
b) Blastic NK cell lymphoma.
c) Mature T and NK cell neoplasm's that has different type which are:
    1. T cell prolymphocytic leukemia.
    2. T cell large granular lymphocytic leukemia.
    3. Aggressive NK cell leukemia.
    4. Extranodal NK/T cell lymphoma, nasal type.
    5. Adult T cell leukemia/ lymphoma.
    6. Enteropathy type Tcell lymphoma.
    7. Hepatospenic T cell lymphoma.
    8. Subcutaneous panniculitis like T cell type.
    9. Mycosis fungoides.
    10. Sezary syndrome.
    11. Primary coetaneous anaplastic large cell lymphoma.
    12. Peripheral Tcell lymphoma, unspecified.
    13. AngioimmunoblasticTcell lymphoma.

## Staging Hodgkin's lymphoma

After doctor has determined the extent of Hodgkin's lymphoma, the cancer will be detecting a stage. Cancer's stage helps to determine prognosis and treatment options (25). Hodgkin's lymphoma may be described as follows (26):

- **A:** The patient does not have fever, weight loss, or night sweats.
- **B:** The patient has B symptoms (fever, weight loss, and night sweats).
- **E:** Cancer is found in an organ or tissue that is not part of the lymph system but which may be next to an involved area of the lymph system.
- **S:** Cancer is found in the spleen.
- The stages of Hodgkin's lymphoma include:
- **Stage I:** In this stage, the cancer is limited to one lymph node region or lymphoid structure or a single organ such as (spleen, thymus, Waldeyers ring) or a single extranodal site (IE).
- **Stage II:** in this stage, the cancer is in two lymph node regions or the cancer invades one organ and extends to the nearby lymph nodes. But the cancer is still limited to a section of the body either above or below the diaphragm.
- **Stage III:** When the cancer extends to the lymph nodes both above and below the diaphragm, it's considered stage III. Cancer may be in one section of tissue or an organ adjacent the lymph node groups or in the spleen.
- **Stage IV:** This is the most advanced stage of Hodgkin's lymphoma. Cancer cells are in several parts of one or more organs and tissue. This stage of Hodgkin's lymphoma affects not only the lymph nodes but also other portion of the body, like the liver, lungs or bone (27).

## Epidemiology

Unlike some other lymphomas, whose incidence increases with age, Hodgkin's lymphoma has a bimodal incidence curve; that is, it occurs most frequently in two separate age groups, the first being young adulthood (age 15–35) and the second being in those over 55 years old although these peaks may vary slightly with ethnic groups (28).

Overall, it is more common in males, except for the nodular sclerosis

variant, which is slightly more common in females. The annual incidence of Hodgkin's lymphoma is about 1 in 25,000 people, and the disease accounts for slightly less than 1% of all cancers world wide (29)**.** In 2010, globally it resulted in about 18,000 deaths down from 19,000 in 1990 (21)**.** The incidence of Hodgkin's lymphoma is increased in patients with HIV infection **(30).** In contrast to many other lymphomas associated with HIV infection it occurs most commonly in patients with higher CD4 T cell counts.

## Epstein-Barr virus and Hodgkin's disease

As early as 1966 MacMahon (31) proposed that Hodgkin's disease might be caused by an infectious agent. The first evidence that this agent might be Epstein-Barr virus (EBV) was provided by the detection of raised antibody titers to EBV antigens in patients with Hodgkin's disease when compared with patients with other lymphomas (32) and, further, that these raised values preceded the development of Hodgkin's disease by several years (33). In addition, the relative risk of developing Hodgkin's disease in individuals with a history of infectious mononucleosis, relative to those with no previous history, was shown to range between 2.0 and 5.0 (34). However, antibody titers to other herpesviruses, including human herpesvirus 6, have been shown to be raised in prediagnostic sera from patients with Hodgkin's disease (35). Although these antibody titers were higher in EBV negative as opposed to EBV positive cases (36). In addition, raised antibody titers to the EBV viral capsid antigen do not predict EBV status in Hodgkin's disease (32). EBV could either play a direct or indirect role in the pathogenesis of Hodgkin's disease, possibly by triggering the pathogenic mechanism(s), or it could reflect the presence of an inherited or acquired depression of immunoregulation that is a prelude both to the malignancy and to the reactivation of EBV(37). Immuno-suppressed patients show rises in all herpes virus antibodies, rather than a selective rise in EBV antibodies (38) which suggests that depression of immune regulation, rather than a specific disease phenomenon, might be responsible for these raised values.

With the advent of cloned viral probes and Southern blot hybridization methods, EBV DNA was initially detected in 20–25% of Hodgkin's disease tumor specimens(39). However, this approach could not determine the locality of the EBV genome in tissues. In situ hybridization methods to detect EBV DNA provided the first demonstration of its existence in the HRS cells(40). Subsequently, the demonstration of the abundant EBV early RNA (EBER1 and EBER2) sequences in HRS cells provided a sensitive method for detecting latent infection in situ. This technique is generally accepted as the “gold standard” for the detection of latent EBV infection in clinical samples (41, 42). However several studies suggest the existence of another form of latency lacking EBER expression(43). Double labeling of malignant Hodgkin-Reed Sternberg (HRS) cells showing co-expression of Epstein-Barr virus early RNAs (EBERs; brown/black) and latent membrane protein 1 (LMP1) (44).

## The role of Epstein-Barr virus in Hodgkin lymphoma

Epstein-Barr virus (EBV) is a ubiquitous herpes virus, which is spread

mainly through saliva between susceptible persons and asymptomatic EBV shedders(33). The majority of primary EBV infections throughout the world are subclinical. Antibodies to EBV have been demonstrated in all population groups with a worldwide distribution; approximately 90 to 95 percent of adults are EBV-seropositive (45).

**Factors suggesting an association**

Early epidemiologic data suggested that HL develops among persons with a delayed exposure to a ubiquitous infectious agent, Epstein-Barr virus (EBV)(46). The following sections will review data suggesting an association between EBV and HL. Mechanisms thought to be involved in the development of EBV-negative HL are presented separately. Association with infectious mononucleosis and EBV — Initial epidemiologic studies that demonstrated an increased risk of HL in patients with a history of infectious mononucleosis were further substantiated by case control studies showing that patients with HL had elevated antibody titers against EBV antigens, which preceded the disease(44).Subsequent studies showed that EBV could be detected in the tumor cells of a subset of patients with HL (47). Finally, in a population-based cohort study of young adults with infectious mononucleosis in Denmark and Sweden, the risk of developing EBV-negative HL after infectious mononucleosis was not increased (relative risk 1.5, 95% CI 0.9-2.5), whereas the risk of developing EBV-positive HL was increased (relative risk 4.0, 95% CI 3.4-4.5), with a median incubation time from mononucleosis to EBV-positive HL of 4 years (95% CI 1.8-8.3 years (46). In this population, the absolute risk of developing HL after infectious mononucleosis was approximately 1 in 1000.

**Genetic loci associated with Hodgkin's lymphoma**

To identify genetic loci associated with susceptibility to classical Hodgkin's lymphoma (cHL) the researchers study confirmed the role of the immune system in susceptibility to cHL, with several associations noted within the HLA region on chromosome 6 (48). Three novel associations on chromosomes 2, 8, and 10 were also identified following a combined analysis of all data including the replication studies, implicating the *REL and GATA3* genes on chromosomes 2 and 10. The region of interest on chromosome 8 contains several genes previously linked with other cancers such as those of the prostate and breast (49).

The researchers conclude that there is a sound biological basis for association between all the genetic regions implicated in their study and risk of cHL. This includes evidence for a relationship between the three novel regions, although the authors were only able to detect nominal interactions between the HLA region and chromosome 2 regions, and between chromosomes 8 and 10 (50,51).

Further studies are needed to investigate possible interactions between these susceptibility loci and their interplay with EBV [Epstein Barr virus] infection" and state that "the modest size of our study makes it likely that further risk variants for cHL can be identified through additional studies. Despite being limited in size, this study has identified several very biologically

interesting susceptibility loci and new insights on the genetic basis of Hodgkin's lymphoma. Although the Epstein Barr virus infection may be causally related to some cases of cHL, the etiology of EBV-negative cases is still largely unknown despite extensive research (52). The findings on chromosomes 2, 6, and 8 in this report were further enriched in EBV-negative cases, which suggests that these regions may be involved in disease etiology via a different pathway from that followed by EBV infection. This could lead to exciting follow-up work (53).

## Genetic Instability in Hodgkin's Lymphoma

Genetic instability is a characteristic feature of the malignant Hodgkin's Reed–Sternberg (HRS) cells. in classical Hodgkin's lymphoma and the lymphocytic and histiocytic (L&H) cells in lymphocyte predominant Hodgkin's lymphoma (19). Genetic instability can be classified into four major categories: (20).

1. Distinct DNA mutations (microsatellite instability).
2. Numerical aberrations (chromosomal instability).
3. Structuralaberrations (translocation instability).
4. And gains or losses of chromosomal regions.

In Hodgkin's lymphoma (HL), HRS cells and L&H cells show somatically mutated clonally rearranged immunoglobulin genes, thus characterizing these cells genetically as germinal center B cells. These cells furthermore show mutations of oncogenes and tumor suppressor genes in some cases (p53, IκBα, CD95/Fas). However, display microsatellite instability, as have a proficient mismatch repair machinery (54). In contrast, HRS and L&H cells frequently harbor recurrent but not specific numerical and structural aberrations as detected by classical cytogenetics and fluorescence in situ hybridization analysis. Results from molecular genetic studies using comparative genomic hybridization and allelotyping (LOH) indicate typical genetic patterns in HL with gains and losses of distinct chromosomal regions (55). In some instances, candidate genes possibly involved in the malignant transformation of HRS cells and L&H cells have been characterized (JAK2, c-REL, MDM2); (Figure 3 and Figure 4) (56).

HRScells, T-cells, granulocytes and histiocytes.

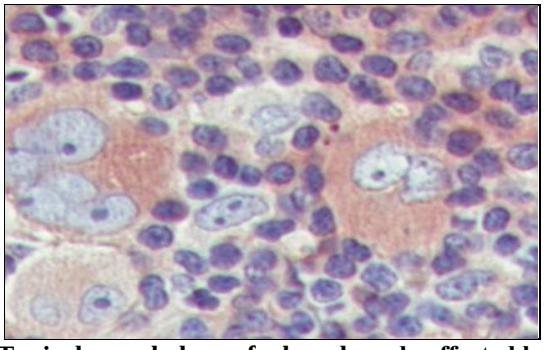

**Figure (3): Typical morphology of a lymph-node affected by Hodgkin's lymphoma. The use of a specific antibody to stain for Hodgkin and Reed-Sternberg cells shows them to be surrounded by a characteristic infiltrate of rosetting T-cells, granulocytes, and histiocytes (19).**

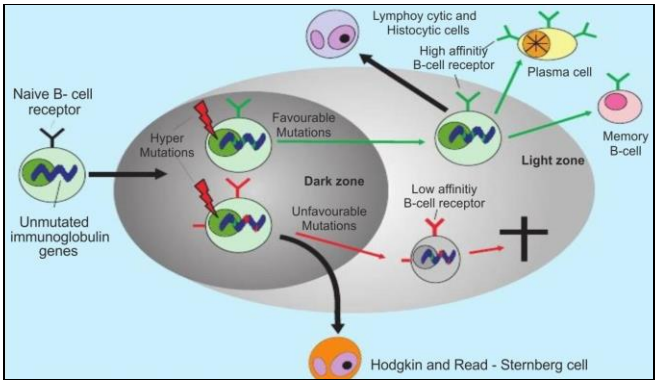


**Figure (4): The germinal-centre derivation of Hodgkin and Reed-Sternberg cells in classic Hodgkin's lymphoma and lymphocytic and histiocytic cells in nodular lymphocyte-predominant Hodgkin's lymphoma (56).**

## Subtle DNA Sequence Changes

Subtle DNA sequence changes, i.e. mutations affecting one or several base pairs, seem to be rare events in HL. For example, analysis of the oncogenes bcl-2 and n-ras in HL showed absence of mutations in most instances (56). Results from the analysis of tumor suppressor genes are somewhat more pro-mising. Somatic mutations were found in genes encoding forIκBα or IκBε in several cases (58)This may be of interest, as these proteins negatively regulate the constitutively activated nuclear factor (NF)-κB expression in HL. Rarely, somatic mutations were also found in the apoptosis controlling genes p53 (58) and Fas/CD95 (60). In B cell- derived HRS cell lines, sequence analysis of bcl-10 did not reveal mutations, while mutations of unknown signifi- cance were detected in the 5′ region of the bcl-6 gene in cell lines (61) as well as in primary cases of HL (62).

The described subtle DNA sequence changes within tumor suppressor genes might be the result of a defect mismatch repair machinery or of a (deregulated) hypermutation machinery. It has been speculated that mutations observed in HL could be the result of a defective mismatch repair (MMR) machinery, which itself might be linked to the hypermutation machinery (63). The human DNA MMR system is responsible for the post replication MMR involving homologs of yeast mutS- and mutL-related proteins (64). In humans, defects in MMR genes have been linked to several solid tumors and hematological malignancies displaying microsatellite instability (MSI) (65). MSI has been analyzed in cHL using a single-cell approach, proving the absence of this form of genetic instability in HRS cells (66). The occurrence of the observed mutations in HL thus might be a by-product of the hypermutation machinery in some instances. could be the result of a defective mismatch repair (MMR) machinery, which itself might be linked to the hypermutation machinery (63).

In accordance with this finding and their GC B cell derivation, it was found that HRS cells express the MMR proteins hMSH2 and hMLH1 (67). It therefore is concluded that HRS cells are MMR- proficient and do not display a mutator phenotype explaining subtle sequence changes in tumor suppressor genes in HL. Alternatively, it has been suggested that there might be a link between the hypermutation machinery

and somatic mutations outside the Ig gene region. The hypermutation machinery is usually site specific (Ig genes), differentiation specific(GC) and lineage specific (B cell) (68). As malignant cells in HL are GC-derived B cells harboring Ig gene mutations, these cells must have been under the influence of the hypermutation machinery at some point in their differentiation. As shown, it is likely that genes other than Ig genes might also be affected by this process (69). The human DNA MMR system is responsible or the post-replication MMR involving homologs of yeast f mutS- and mutL-related proteins (64, 70).

## Chromosomal Instability

Chromosomal instability is defined as the occurrence of numerical chromosomal aberrations. In hereditary non-polyposis colorectal cancer, this form of genetic instability correlates inversly with MSI. It is therefore not surprising that the MMR proficient multinuclear Reed–Sternberg cells typically show a grossly abnormal karyotype, including gains and losses of whole chromosomes. Although classical cytogenetics has been used in the analysis of HRS cells for decades, there are at best several hundred metaphases that can be evaluated (71).

Moreover, results from these studies show a large variability regarding the percentage of abnormal metaphases (ranging from 13 to 92%), suggesting that the majority of HRS cells are diploid. If abnormal karyotypes were observed in HRS cells, gains would be observed more frequently than losses, with the exception of chromosomes 13, 15, 22 and Y. In these cases, gains were found for all chromosomes with gains of chromo somes 12 and X being detected in almost every other HL case (72) .

In contrast to the classical cytogenetic approach, a study performing fluorescence in situ hybridization (FISH) in com bination with immunohistochemistry (FICTION) revealed a more uniform picture: all HRS cell karyotypes analyzed showed numerical chromosome aberrations in the hyper diploid range (73). It has therefore been concluded that chromosomal instability is a characteristic feature of malignant cells in HL. The mechanisms leading to chromosomal instability in HRS cells are not yet understood. Recently, there have been two studies showing that cell fusion is unlikely to explain polyploidy in HRS cells (30). It might be speculated that polyploidy is the result of a deregulated differentiation process of the HRS cells or of an altered expression of mitotic spindle checkpoint genes (‘endomitosis’).

## Chromosomal Translocations

The simple type of translocation is observed frequently in leukemias and non-Hodgkin’s lymphoma. This type includes distinct translocations that are typically and reproducibly found in the neoplastic cells. One example of such a simple translocation is t(14;18)(q32;q21), involving the B cell lymphoma/leukemia (bcl-2) locus in follicular center B cell lymphomas. Another example is t(2;5)(p23;35), involving the anaplastic lymphoma kinase (alk) and nucleophosmin genes in anaplastic large-cell lymphoma. Neither the t(14;18), nor the t(2;5) nor other known simple translocations are characteristic for HL (74, 75). It is therefore concluded that in HL there are

no known specific simple genetic aberrations that would appear to be necessary for the malignant transformation. As in HL, the occurrence of complex marker chromosomes involving several chromosomes is observed frequently. It is therefore conceivable that these complex alterations mask important simple translocations. In HRS and L&H cells, complex structural chromosome aberrations have been described in numerous classical cyto genetic studies as well as in FISH analyses, and some of these aberrations are detected recurrently. Among these recurrent changes, alterations of chromosomes 2p, 3q, 6q, 7q, 9p, 13p, 14p and 17q are found more frequently than expected. Several breakpoints are detected non-randomly in HL, including3q27, 6q15, 7q22, 11q23, 14q32 (76) , but translocation partners have not yet been identified. Since several of these alterations have been described in HRS cell lines as well, it might be that a more precise genetic analysis of these cell lines using modern molecular genetic tools will help identification of pathogenetically relevant genes(Figure 5 ) (77).

## Gene Amplification and Deletion

Recently, the application of sophisticated molecular techniques, including the micromanipulation of single HRS and L&H cells, allowed the genetic analysis of neoplastic cells in HL(69). Tumor DNA was isolated from single or pooled cells, pre-amplified in some instances and analyzed for genetic imbalances using genomic comparative hybridization (CGH) or loss of heterozygosity (LOH). All but one study included only cases of cHL. One study analyzing LPHL with CGH found complex chromosome aberrations with gains of chromosomes 2q, 4q, 5q, 6 and 11q, which might be a characteristic feature of LPHL since these aberrations are rarely observed in other lymphomas (78).

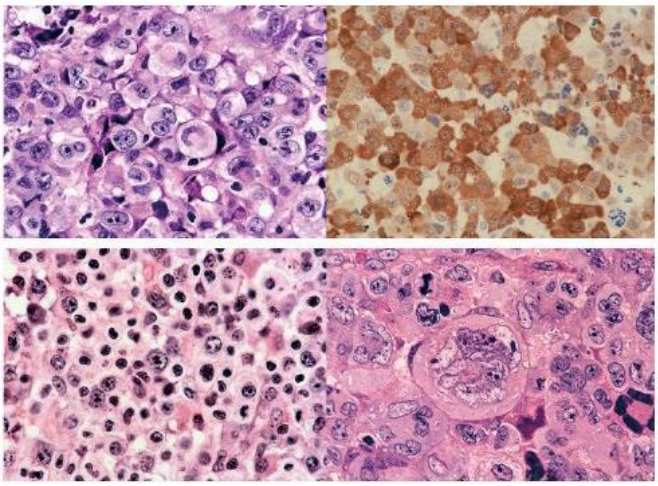

**Figure (5):** A (upper left); B (upper right); C (lower left), and D (lower right).Anaplastic lymphoma kinase (ALK)-positive anaplastic large cell lymphoma (ALCL). **A.** Common type of ALK+ ALCL showing predominant population of large cells with irregular nuclei (HE ×200). Note large cells ("hallmark" cells) with eccentric kidney-shaped nuclei (×125). **B.** Tumor cells positive for ALK in their cytoplasm and nuclei. **C.** Small cell variant of ALK+ ALCL showing predominant population of small cells with irregular nuclei and admixture of "hallmark" cells (HE ×200). **D.** Giant cell-rich variantof ALK+ ALCL consisting of pleomorphic giant cells (HE ×200)(75).

As mentioned, most investigators have focused on the genetic analysis of HRS cells in cHL. So far, two groups have presented their CGH results, primarily looking for amplifications of oncogenes (79) detected recurrent gains on chromosomes 2p, 9p and 12q with high level amplifications on 4q16, 4q23–q24 and 9p23–p24. FISH studies performed with these samples led to the identification of gene amplifications, i.e. amplifications of the JAK2 gene on 9p23–p24 and the MDM2 gene on 12q14 (12). As reported at the fifth International Symposium on Hodkgin's Lymphoma in Cologne (Germany) in September 2001, Barth (80) found an amplification of the c-Rel locus on chromosome 2p,which might be one explanation for the constitutive activity of NF-κB in HRS cells. This pathway seems to be specific for cHL of the nodular sclerosis subtype (81).

Independently, a second group described gains on 1p13 and 7q35–q36 and losses on16q11–q21 using CGH (82). A detailed mapping of the chromosomal arm 16q by LOH identified E-cadherin as a candidate gene involved in tumorigenesis. Rather than CGH, two groups have used LOH as a screening tool for inactivation of tumor suppressor genes (66). Analyzed seven cases using four markers and found alterations on chromosomes 1q42, 4q26, 9p23 and 11q22– q23. performed an LOH study analyzing 16 cases of cHL using 30 markers. A high degree of monoallelic losses, especially on the chromosome arm 6q24–q25, found to be a characteristic feature of other B cell lymphomas (83). Candidate tumor suppressor genes located within the mentioned chromosomal region are currently being analyzed.

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