Histopathologic and Immunocytochemical Study of Hodgkin's Disease

Soo Im Choi, Kyu Rae Kim, Hyeon Joo Jeong, Chan Il Park
and In Joon Choi

Hodgkin's disease primarily involves the lymphoreticular system with characteristic tumor cells and infiltration of reactive lymphocytes, eosinophils and plasma cells. The present study investigates the incidence of the disease by subtypes and by geographic differences, its clinicopathologic features and properties of the tumor cells. Fifty-eight cases were retrospectively reviewed, and the results were as follows; 1) Histopathologic classification by the Rye modification were; lymphocyte predominance (LP) 4 cases, nodular sclerosis (NS) 12 cases, mixed cellularity (MC) 26 cases and lymphocyte depletion (LD) 16 cases 2) The geographic difference in the distribution of subtypes of Hodgkin's disease between the Seoul and Wonju areas was noted and in Seoul, the ratio of NS was higher than in Wonju, and the ratio of LD was higher in Wonju than in Seoul 3) Age distribution was between 6 and 70 years with a mean age of 37 years. The peak incidence was seen between the 3rd and 5th decades. The male to female ratio was 2.9:1 with 43 male patients and 15 female 4) Utilizing the Ann Arbor staging method, 75.87.5% of LP, MC and NS were classified as stage I or II and 81.3% of LD were stage III or IV 5) The initial biopsy sites most commonly seen were in the peripheral lymph nodes (54 cases) and one case each in the mediastinal lymph node, thymus, tonsil and stomach 6) The most frequent initial chief complaint was a palpable mass (60.3%), other complaints included fever, cough, dyspnea, anorexia, general weakness and hematemesis 7) Among 58 cases, 32 cases were studied using an immunoperoxidase stain. The Reed-Sternberg (R-S) cells and/or Hodgkin's cells were positive for Kappa and Lambda light chains, simultaneously. One case was positive for lysozyme and all were negative for S-100 protein. For the α1-antichymotrypsin, 13 cases exhibited a positive reaction. These findings suggest that R-S cells might be the histiocytic origin.

Key Words: Hodgkin's disease, Reed-Sternberg cell, histiocytic origin

Hodgkin's disease was described first by Thomas Hodgkin in 1832 and many studies have been done concerning its etiology, classification, treatment and prognosis.

It was classified as paragranuloma, granuloma, and sarcoma according to Jackson and Parker (1847). In 1963, Lukes and Collins suggested a new classification which was modified at the Rye conference and used until now (Lukes 1966). At that conference, Hodgkin's disease was classified as lymphocyte predominance (LP), nodular sclerosis (NS), mixed cellularity (MC), and lymphocyte depletion (LD). It is accepted that classification has a relationship with prognosis while a classification of non-Hodgkin's lymphoma is controversial because of a lack of correlation with clinical courses.

Although much progress had been made in the classification, treatment and prognosis of this disease, the etiology and the origin of the tumor cell are in much controversy. With respect to etiology, association with the Epstein-Barr virus has been suggested (Johanson et al. 1970; Levine et al. 1971), and recently, the environmental, genetic or immunological aspects have been reported. Because the distribution of incidence, age and histological type is different between advanced, developing and underdeveloped countries (Correa et al. 1971) and the Epstein-Barr virus titer of patients is no more increased than the control group (Goldman et al. 1970), many cases are associated with a specific HLA-antigen (Bodmer 1973) and various immunological defects are found in the patients (Romagnani et al. 1985). Although the exact nature of the Reed-Sternberg (R-S) cells is not defined.
due to difficulties in the culture of tumor cells, many studies have utilized cytochemical (Beckstead et al. 1982; Carbone et al. 1983), immunochemical (Kadin et al. 1986), cell culture (Kaplan 1977; Gang et al. 1979; Roberts 1978), and electron microscopic examination (Glick et al. 1976; Anagnostou et al. 1977). They suggested B-lymphocyte, T-lymphocyte, histiocyte or macrophage as the origin of the R-S cell.

The purpose of this study is to note the difference in clinical manifestation and incidence between the subtypes classified according to the Rye modification and to determine the immunocytochemical characteristics and origin of the R-S and Hodgkin's cells.

MATERIAL AND METHODS

Material

Fifty-eight cases were reviewed which had been diagnosed as Hodgkin's disease in the Department of Pathology, College of Medicine, Yonsei University, Youngdong Severance Hospital and Wonju College of Medicine, Yonsei university, during a 10 year period (January 1977 to December 1986).

Methods

Clinical records were reviewed for the age, sex, chief complaint, sites of involvement, clinical stage, and complication. For the light microscopic examination, the specimens were fixed in 10% neutral formalin and embedded in paraffin. They were sectioned in 5 um thickness and stained by hematoxylin eosin. Reticulin, methyl-green pyronine or trichrome stains were done if needed. They were examined by light microscope and classified according to the Rye modification. For the immunocytochemical studies the followings were used, peroxidase-antiperoxidase stain for the Kappa and Lambda light chains of the immunoglobulin as a surface marker for B-lymphocytes; and lysozyme, S-100 protein and α1-anti-chymotrypsin for histiocytes.

RESULTS

Histopathological subtypes of Hodgkin's disease

26 cases (44.8%) were MC, followed by LD (16 cases, 27.6%), NS (12 cases, 20.7%) and LP (4 cases 6.9%) (Table 1).

<table>
<thead>
<tr>
<th>Type</th>
<th>Male</th>
<th>Female</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte predominance</td>
<td>4</td>
<td>0</td>
<td>4 ( 6.9)</td>
</tr>
<tr>
<td>Nodular sclerosis</td>
<td>7</td>
<td>5</td>
<td>12 (20.7)</td>
</tr>
<tr>
<td>Mixed cellularity</td>
<td>17</td>
<td>9</td>
<td>26 (44.8)</td>
</tr>
<tr>
<td>Lymphocyte depletion</td>
<td>15</td>
<td>1</td>
<td>16 (27.6)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>43</td>
<td>15</td>
<td>58 (100.0)</td>
</tr>
</tbody>
</table>

Table 2. Geographic differences of histologic subtype of Hodgkin's disease

<table>
<thead>
<tr>
<th></th>
<th>LP (%)</th>
<th>NS (%)</th>
<th>MC (%)</th>
<th>LD (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seoul</td>
<td>3 (7.1)</td>
<td>10 (23.8)</td>
<td>19 (45.2)</td>
<td>10 (23.8)</td>
<td>42 (100)</td>
</tr>
<tr>
<td>Wonju</td>
<td>1 (6.3)</td>
<td>2 (12.6)</td>
<td>7 (43.8)</td>
<td>6 (37.5)</td>
<td>16 (100)</td>
</tr>
</tbody>
</table>

LP: lymphocyte predominance NS: nodular sclerosis MC: mixed cellularity LD: lymphocyte depletion

Table 3. Age distribution by histological type (n=58)

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>LP</th>
<th>N S</th>
<th>M C</th>
<th>L D</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 10</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>6 (10.3)</td>
</tr>
<tr>
<td>11 - 20</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>4 (6.8)</td>
</tr>
<tr>
<td>21 - 30</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>13 (22.4)</td>
</tr>
<tr>
<td>31 - 40</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>10 (17.2)</td>
</tr>
<tr>
<td>41 - 50</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>12 (20.7)</td>
</tr>
<tr>
<td>51 - 60</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>6 (10.3)</td>
</tr>
<tr>
<td>61 - 70</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>4 (6.8)</td>
</tr>
<tr>
<td>Mean age</td>
<td>33</td>
<td>31</td>
<td>37</td>
<td>44</td>
<td>37 (100.0)</td>
</tr>
</tbody>
</table>

LP: lymphocyte predominance NS: nodular sclerosis MC: mixed cellularity LD: lymphocyte depletion

Geographic differences of histologic subtypes of Hodgkin's disease

Between the Seoul and Wonju areas, the percentage of NS which has better prognosis, was higher in Seoul (23.8%) than in Wonju (12.6%), and LD which has a poor prognosis, was more in Wonju (37.5%) than in Seoul (23.8%) (Table 2).

Age distribution by histological type

The age of the patients was between 6 and 70
years. Among them, 43 were male and 15 were female with a male:female ratio of 2.9:1. 13 (22.4%) were between 21 and 30 years and 12 (20.7%) were between 41 and 50 years. 60% were in between 21 and 50 years old. The mean age of each subtype were 33 years in LP, 37 years in MC, 44 years in LD and 31 years in NS (Table 3).

Sites of involvement

Initial biopsy sites were lymph nodes in 54 cases, the mediastinum in 2 cases, and each of the thymus, tonsil and stomach (Table 4). In 17 cases, further involvement was confirmed by exploratory laparotomy or biopsy, and these sites were the spleen (6), abdominal lymph nodes (4), mediastinal lymph nodes (1) and the liver (2) (Table 5).

Initial symptoms of Hodgkin’s disease

Initial symptoms of Hodgkin’s disease were enlargement of peripheral lymph node (60.3%), followed by fever (32.6%), cough (5.2%), and 1 case each of dyspnea, anorexia, general weakness and hematemesis (Table 6).
Table 9. Immunocytochemical properties of R-S and/or Hodgkin's cells

<table>
<thead>
<tr>
<th>No. of cases examined</th>
<th>Kappa</th>
<th>Lambda</th>
<th>Lysoyme $\times 100$</th>
<th>$\alpha_1$-ACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>NS</td>
<td>9</td>
<td>9</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>MC</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>LD</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Total (%)</td>
<td>32</td>
<td>18(56.2)</td>
<td>18(56.2)</td>
<td>1(3.1)</td>
</tr>
</tbody>
</table>

LP: lymphocyte predominance  NS: nodular sclerosis
MC: mixed cellularity  LD: lymphocyte depletion
$\alpha_1$-ACT: $\alpha_1$ antichymotrypsin

Complications of Hodgkin's disease

Infection was the most common complication of Hodgkin's disease and among the patients there were 4 cases of pneumonia, 3 cases of sepsis, and 3 cases of herpes zoster infection. One patient died of hepatic necrosis (Table 8).

Immunocytochemical properties of R-S and/or Hodgkin's cells

Thirty-two cases were studied using an immunoperoxidase stain. Against B-lymphocytic markers, 18 cases (56.2%), which included 1 case of LP, 6 cases of MC, 8 cases of LD and 6 cases of NS were positive for both Kappa and Lambda light chains, simultaneously. Against $\alpha_1$-anti-Chymotrypsin, 13 cases (40.6%) which included 5 cases of MC, 5 cases of LD, and 3 cases of NS exhibited a positive reaction (Table 9).

DISCUSSION

Hodgkin's disease involves mainly the immune system and presents characteristic tumor cells with infiltrations of reactive lymphocytes, eosinophils and plasma cells. Recently, much progress has been made concerning histological subtyping, treatment and prognosis.

The Lukes and Butler classification (Lukes 1966) which was modified at the Rye conference is used to classify this disease, and the distribution of subtypes is different throughout the world (Ioachim 1975). In Europe, MC and NS are more common (Baroni et al. 1980; Desforges et al. 1979; Agnarsson et al. 1987) and it is the same in the USA and Japan (O'Connor et al. 1973; Wakasa 1973). In Africa MC and LD are more common (Wright 1973) and in Korea, MC was the most common followed by LD (Lee et al. 1973; Shin et al. 1983) and LP (Kim et al. 1982; Choi et al. 1984) (Table 10).

Correa and O'Connor (1971) classified the distribution of Hodgkin's disease into 3 types. In underdeveloped countries, a younger age group and types with a poor prognosis were more common, and in advanced countries, a young age group and types with a better prognosis were common. Korea is similar to Africa in that LD is the commonest.

With regard to the distribution, it is reported that it has 'bimodal curve' which has two peaks in the second decade and the 4th and 5th decades (MacMahon 1966; Stalsberg 1973; Shin et al. 1983; Kim et al. 1982). But several reports do not show this bimodal curve (Colby et al. 1981; Baroni et al. 1980; O'Connor et al. 1973). In this study, a typical bimodal curve is not noted, and 60.3% is seen in the third to 5th decades with a relatively low incidence in the second decade. The mean age of LD was 44 and that of NS was 31 which means that the type with a better prognosis occurs in the younger age group than the type with a poor prognosis.
The initial symptom of Hodgkin's disease was enlargement of a superficial lymph node in 60.3%. The remainder includes fever, cough, dyspnea, general weakness and hematemesis.

The primary site of involvement in Hodgkin's disease is generally the immune system, but it also may metastasize to the gastrointestinal tract, bone marrow or liver. In this study, involvement of peripheral lymph node was the commonest (54 cases) and other sites were mediastinal lymph node, thymus, tonsil and gastrointestinal tract. 17 cases, in which involvement was confirmed by exploratory laparotomy or biopsy, included MC (10 cases) or LD (7 cases) and the most common site was the spleen (6 cases).

In Hodgkin's disease, both the histological type and the clinical stages are associated with prognosis (Chawla et al. 1970). In this study, 75% of LP and NS, which have a better prognosis, were stage I or II and 81.3% of LD were stage II or IV. In stage I and II the absence of B-symptoms such as fever, weight loss and night sweats was more common than the presence, and higher the stage, the more the presence of B-symptoms.

In Hodgkin's disease, various immunological defects, especially in cell mediated immunity, cause a disturbance of delayed hypersensitivity, which leads to infections by mycobacterium, histoplasma, cryptococcus, herpes or toxoplasma (Romagnani et al. 1985). These defects of cell mediated immunity are thought to come from increased activity of helper T-lymphocytes and B-lymphocytes by an overstimulation of a certain antigen which is not known yet (Kaplan 1980). In this study, 11 cases were complicated by infection, among those, 3 were herpes zoster and 4 were bacterial pneumonia; other opportunistic infections were not seen.

Among the efforts to clarify the causes of Hodgkin's disease, many studies have looked at the nature of the R-S and Hodgkin's cells which are known to be the neoplastic cells in Hodgkin's disease. About the origin of these cells, many suggestions are made such as T-lymphocyte (Stein et al. 1986), B-lymphocyte (Stuart et al. 1983; Pinkus et al. 1985), macrophage (Dorfman et al. 1986; Kadim et al. 1978), histiocyte (Pinkus et al. 1985; Curran et al. 1978; Mir et al. 1983) or reticulum cell (Beckstead et al. 1982; Hsu et al. 1986; Kadim 1982).

Histochemically, R-S cells are weakly positive to acid phosphatase and nonspecific esterase in the perinuclear region resembling reticulum cells, but are not identical with them because they are negative for ATPase (Beckstead et al. 1982; Carbone et al. 1983). Electron microscopy of the R-S cells reveals large nuclei and nucleoli with many polyribosomes in the cytoplasm like lysosomal granules and microfilament (Glick et al. 1976; Anagnostou et al. 1977). Garvine (1974) suggested a B-lymphocytic origin. But the immunoglobulins were interpreted as phagocytosed ones because they showed polyclonality and others said that the origin was histiocytes (Kadin et al. 1978; Chang et al. 1984).

In studies using cell culture, R-S cells have nonspecific esterase, phagocytose immunoglobulin, contain Fc receptor, and exhibit positive reaction to lysozyme, and thus a histiocytic origin was suggested (Kaplan et al. 1980). Some authors agree that the R-S cells are not T-lymphocytes because they don't have the surface antigen of T-lymphocytes and the E-rosette receptor (Poppema et al. 1982).

In the LP subtype of Hodgkin's disease, the origin of the R-S cell may be different from other types, because of positivity for LN-1 and negativity for Leu-M1 (Pinkus et al. 1985; Trudel et al. 1987; Stein et al. 1986; Timens et al. 1986). But Hsu et al. (1986) reported that they were positive for Leu-M1 after treatment with neuraminidase and this result strongly suggests a histiocytic origin like other subtypes.

Kadin (1982) and Beckstead et al. (1982) said that the R-S cells are reticulum cells rather than monocytes/macrophages, because of the morphology, location in the lymph node and association with helper T-lymphocytes. Reticulum cells, a kind of histiocyte, are negatively stained by lysozyme and α1-anti-chymotrypsin and positively by S-100 protein and are present in the T-zone in lymph nodes. Therefore these are different from the monocytes/macrophages. But R-S cells are negative for lysozyme and α1-anti-chymotrypsin (Mir et al. 1983). In our study, R-S cells were negative for S-100 protein and 40.6% were positive for α1-anti-chymotrypsin. Because 18 cases, which were positive for Kappa and Lambda light chain, showed polyclonality, these findings suggest that R-S cells might be of histiocytic origin.

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