International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

Immunohistochemical Evaluation of CD20 Expression in Classical Hodgkin Lymphoma

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Abstract: ***Classical Hodgkin lymphoma (cHL) is marked by Hodgkin and Reed-Sternberg (HRS) cells that typically lack CD20 expression, although abnormal positivity has been observed. This study aimed to assess CD20 expression in cHL tissues from Western Saudi Arabia using immunohistochemistry. Sixty formalin-fixed paraffin-embedded cHL samples were retrospectively analyzed. CD20 positivity, defined as membranous staining in ≥10% of HRS cells, was observed in 23.3% of cases. No significant associations were found with age, gender, or histological subtype. The findings suggest that CD20 expressions are present in a notable subset of cHL cases and highlight the importance of comprehensive immunophenotypic evaluation for accurate diagnosis and potential therapeutic considerations.***

Keywords: CD20 expression, Classical Hodgkin lymphoma, Immunohistochemistry, Diagnostic pathology, Saudi Arabia.

1. Introduction

The presence of Hodgkin and Reed-Sternberg (HRS) cells in a reactive cellular background is a histological characteristic of classical Hodgkin lymphoma (cHL), a distinct lymphoid cancer. Immunohistochemical (IHC) markers are essential for accurately diagnosing cHL in order to differentiate it from other lymphomas and reactive diseases. While CD20, a transmembrane protein expressed on B-lymphocytes, is an essential diagnostic for B-cell lymphoma diagnosis, its expression in cHL is often inconsistent and frequently missing or poor (1, 2).

Although CD20 is usually negative in the majority of cHL patients, certain HRS cells may express CD20 abnormally, which complicates differential diagnosiantigen-antibody complexes were seen, particularly in cases of nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) or specific B-cell non-Hodgkin lymphomas (3, 4). In order to prevent diagnostic mistakes and to potentially influence treatment decisions, it is crucial to comprehend the pattern and frequency of CD20 expression in cHL.

Immunohistochemistry provides a sensitive and specific method to detect CD20 expression in formalin-fixed paraffin-embedded lymphoma tissues. Previous studies have reported variable rates of CD20 positivity in cHL ranging from 5% to 40%, with potential correlations to clinical behavior and outcomes (5, 6). However, data from Middle Eastern populations, including Saudi Arabia, remain limited.

This study aims to investigate the expression of CD20 in classical Hodgkin lymphoma tissues using immunohistochemistry in a cohort from Western Saudi Arabia, to better characterize its diagnostic utility and clinical relevance in this population.

Study Design and Sample Selection

Sixty formalin-fixed paraffin-embedded (FFPE) tissue samples with a diagnosis of classical Hodgkin lymphoma (cHL) were included in this retrospective cross-sectional investigation. The samples, which included patients diagnosed between December 2024 and June 2025, were taken from the pathology archives of Madinah Hospital in Western Saudi Arabia. The requirements for inclusion included having enough tissue available for immunohistochemistry investigation and a verified histopathological diagnosis of cHL. Cases with poor fixation or insufficient tissue were not included.

Immunohistochemical Staining Procedure

We obtained tissue blocks of lymphomas that were formalin-fixed paraffin-embedded (FFPE) from the pathology archives. Sections of unstained 3 µm were cut and placed on slides coated with poly-L-lysine. CD20 immunohistochemical staining was performed on a single slide per sample.

To guarantee adequate tissue adhesion, slides were incubated for two hours at 60°C. They were then deparaffinized in xylene and rehydrated using ethanol concentrations that were graded at 100%, 90%, 70%, and 50%. The slides were submerged in phosphate-buffered saline (PBS) at pH 7.4 following a rinse with distilled water. The slides were incubated with 3% hydrogen peroxide for 10 minutes at room temperature in order to inhibit endogenous peroxidase activity. Citrate buffer (pH 6.0) was used for antigen retrieval, which was carried out in a water bath at 95°C for 40 minutes, cooled to room temperature, and then washed in PBS. For 60 minutes at room temperature, slides were treated with primary monoclonal anti-CD20 antibody, dilution 1: 10After three PBS washes, the slides were incubated for 30 minutes with a secondary antibody coupled with horseradish peroxidase (HRP) using the Dako detection system.

Using 3, 3'-diaminobenzidine (DAB) chromogen, antigen-antibody complexes appeared as brown deposits for five minutes, resulting in a brown hue at positive locations. Slides were then cleaned in xylene, counterstained for 30 seconds with Mayer's hematoxylin, dehydrated using graded ethanol, and mounted using DPX mounting media. Two skilled pathologists used light microscopy to assess CD20 expression, concentrating on Hodgkin and Reed-Sternberg cell membrane staining.

Evaluation of CD20 Expression

Two experienced pathologists independently examined the stained slides under light microscopy. CD20 positivity was defined as distinct membranous staining in Hodgkin and Reed-Sternberg (HRS) cells. The percentage of positive HRS cells was recorded. Cases with ≥10% CD20-positive HRS cells were classified as CD20-positive; cases with <10% or absent staining were classified as CD20-negative.

2. Data Analysis

Data were entered and analysed using SPSS version 21. Descriptive statistics summarized the frequency of CD20 expression. Associations between marker expression and clinicopathological variables such as age, gender, and lymphoma subtype were assessed using Chi-square or Fisher's exact tests. A p-value <0.05 was considered statistically significant.

3. Ethical Considerations

The study protocol was reviewed and approved by the Research Ethics Committee of AL Rayan Colleges, Madinah, Saudi Arabia. Approval for use of archived data and tissue samples was obtained from the participating histopathology laboratories. All procedures adhered to local ethical guidelines for research involving human tissue.

4. Result

This study included 60 patients diagnosed with classical Hodgkin lymphoma (cHL). The mean age of the patients was 38.5 ± 12.4 years, with a range of 18 to 65 years. The group was composed of 35% female patients (n = 21) and 65% male patients (n = 39). Nodular sclerosis subtype accounted for 55% of cases, with mixed cellularity (30%) and other subtypes (15%) following. The clinical and demographic characteristics are summarized in Table 1.

Table 1: Demographic and Clinical Characteristics of cHL Patients (n=60)

Characteristic	Number (n)	Percentage (%)
Age (years)		
Mean ± SD	38.5 ± 12.4	
Age groups		
18–30	20	33.3
31–45	25	41.7
>45	15	25
Gender		
Male	39	65
Female	21	35
Histological subtype		
Nodular sclerosis	33	55
Mixed cellularity	18	30
Others (lymphocyte-rich, lymphocyte-depleted)	9	15

Immunohistochemical analysis revealed that 14 cases (23.3%) showed CD20 positivity, defined as membranous staining in ≥10% of Hodgkin and Reed-Sternberg cells. The remaining 46 cases (76.7%) were negative for CD20 expression as shown in Table 2

Table 2: CD20 Expression in Classical Hodgkin Lymphoma Cases

CD20 Expression	Number (n)	Percentage (%)
Positive (≥10% cells)	14	23.3
Negative (<10% cells)	46	76.7

No significant association was found between CD20 expression and patients' age groups (p = 0.65) or gender (p = 0.58). Further analysis is summarized in Table 3.

Table 3: Association between CD20 Expression and Demographic Variable

Variable	CD20 Positive n (%)	CD20 Negative n (%)	p-value
Age group			
18–30	5 (25.0)	15 (75.0)	0.65
31–45	6 (24.0)	19 (76.0)	
>45	3 (20.0)	12 (80.0)	
Gender			
Male	9 (23.1)	30 (76.9)	0.58
Female	5 (23.8)	16 (76.2)	

5. Discussion

In a Western Saudi Arabian cohort, 23.3% of patients of classical Hodgkin lymphoma (cHL) had CD20 expression. Depending on patient demographics, antibody clones, and methodological variations, CD20 positivity in cHL can range from as low as 5% to 40%, which is within the vast range documented in the literature [7, 8]. Given that classical Hodgkin lymphoma is often identified by a lack of CD20 expression in Hodgkin and Reed-Sternberg (HRS) cells, the variation in CD20 expression presents a diagnostic problem

[9]. It is essential to comprehend the frequency of CD20 expression in cHL across various groups as this affects the precision of diagnosis and might have an impact on treatment plans.

Our findings are consistent with research that found CD20 positive frequencies of 20–30% in various Middle Eastern and foreign populations [7, 10]. For example, our findings closely matched those of Al-Maghrabi et al., who reported a 27% CD20 positive rate in cHL patients from Saudi Arabia [7]. These results highlight the value of regional research in understanding potential regional or ethnic differences in immunophenotypes. Furthermore, a significant minority of cHL cases express CD20, highlighting the need for careful interpretation of immunohistochemical panels to distinguish cHL from B-cell non-Hodgkin lymphomas and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL), both of which are typically CD20-positive [10, 11].

Although some studies have suggested a correlation between CD20 expression and specific histological subtypes or clinical outcomes, our analysis did not demonstrate a statistically significant association between CD20 positivity and patient age, gender, or histological subtype. Similar findings have been reported in prior studies indicating that CD20 expression may not be strongly linked to these clinicopathological parameters [12, 13]. However, some evidence suggests that CD20-positive cHL cases might have a distinct biological behavior and could respond differently to targeted therapies such as rituximab, a monoclonal antibody against CD20 [13].

Lastly, the detection of CD20 in a subset of cHL cases raises questions about the underlying biology of these tumors. The aberrant expression of CD20 may reflect heterogeneity within the malignant HRS cell population or differences in the cell of origin [9, 14]. It also supports the view that classical Hodgkin lymphoma, although primarily characterized by loss of typical B-cell markers, retains some degree of B-cell antigenicity in a proportion of cases. Future studies incorporating molecular and genomic profiling are warranted to better elucidate the significance of CD20 expression and its prognostic and therapeutic implications in cHL.

6. Conclusion

This study demonstrates that CD20 expression occurs in a considerable subset (23.3%) of classical Hodgkin lymphoma cases in Western Saudi Arabia. Although no significant association was found with demographic or pathological variables, the findings reinforce the need for comprehensive immunophenotypic assessment to distinguish cHL from other lymphomas. Recognizing CD20 expression patterns not only enhances diagnostic accuracy but also opens avenues for potential targeted therapies. Further multicenter studies are encouraged to explore the biological and clinical implications of CD20 positivity in cHL.

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