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# Immunohistochemical Expression of Signal Transducer and Activator of Transcription in Classic Hodgkin Lymphoma

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**Disclaimers**

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|---|---|
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## ABSTRACT

**Background**: Hodgkin lymphoma (HL) is a significant malignancy, with Classic Hodgkin Lymphoma (CHL) and Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL) being its main subtypes. STAT6, a transcription factor in the IL-4/IL-13 pathway, may aid in distinguishing between these subtypes through immunohistochemical analysis.

**Objective**: To evaluate the diagnostic utility of STAT6 immunohistochemical expression in differentiating CHL from NLPHL.

**Methods**: A comparative cross-sectional study was conducted on 94 lymphoma cases, including 53 CHL and 41 NLPHL cases. Tissue samples were fixed, processed, and stained with STAT6 antibody. Nuclear and cytoplasmic STAT6 expression was assessed in neoplastic cells. Statistical analysis was performed using SPSS version 25, with Fisher's exact test applied.

**Results**: Nuclear STAT6 expression was observed in 100% of CHL cases (53/53) and in 24.39% of NLPHL cases (10/41). Combined nuclear and cytoplasmic STAT6 expression was present in all CHL cases (53/53) but absent in NLPHL cases (0/41).

**Conclusion**: STAT6 nuclear expression serves as a reliable marker to distinguish CHL from NLPHL, with a 100% negative predictive value for NLPHL.

## INTRODUCTION

Hodgkin lymphoma (HL) is a significant hematological malignancy observed globally, with particular prevalence in both developed and developing countries. In Pakistan, it accounts for approximately 4.9% of all reported cancer cases (1). HL is generally classified into two main subtypes: Classic Hodgkin Lymphoma (CHL) and Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL), both originating from germinal center B-cells. These subtypes exhibit distinct clinical and pathological features. CHL is characterized by the presence of Reed–Sternberg cells (RSCs) within a reactive cellular background, consisting predominantly of non-neoplastic immune cells, including T cells, B cells, eosinophils, neutrophils, and macrophages (2-4). The clinical presentation of CHL typically involves classic B symptoms such as fever, night sweats, and weight loss, along with lymphadenopathy involving multiple sites. Conversely, NLPHL presents with more localized lymphadenopathy, often without B symptoms, and generally follows a more indolent course (5). Diagnosis of CHL is primarily reliant on identifying RSCs in hematoxylin and eosin-stained sections, supported by immunohistochemical markers such as CD30, CD15, and Pax5, among others. However, due to the heterogeneity in the expression of these markers within the same tumor and across different patients, challenges in diagnosis persist (6-8). NLPHL, on the other hand, is diagnosed through the identification of lymphocyte-predominant (LP) cells, which express a different immunophenotypic profile, notably CD20 positivity and CD30/CD15 negativity, with considerable morphological overlap with RSCs (9).

The pathogenesis of HL is strongly linked to aberrant cytokine signaling, particularly involving the interleukin-13 (IL-13) pathway, which is frequently upregulated in CHL. IL-13, secreted by RSCs, contributes to the unique tumor microenvironment of CHL, characterized by a diverse infiltrate of reactive immune cells (10). Signal Transducer and Activator of Transcription 6 (STAT6) is a critical mediator in the IL-4/IL-13 signaling pathway, becoming activated through phosphorylation by Jak2, which subsequently leads to its dimerization and translocation to the nucleus, where it influences gene transcription (11). Given the importance of the JAK-STAT pathway in HL, there has been considerable interest in exploring the diagnostic utility of STAT6 in differentiating between CHL and NLPHL. The present study aims to evaluate the immunohistochemical expression of STAT6 in neoplastic RSCs of CHL and LP cells of NLPHL, with the goal of establishing its potential as a diagnostic marker. In particular, we sought to determine whether nuclear expression of STAT6 could serve as a reliable indicator for CHL, thereby facilitating its distinction from NLPHL in challenging cases where conventional markers may be inconclusive.

## MATERIAL AND METHODS

This study employed a comparative cross-sectional design conducted at the Department of Histopathology, Armed Forces Institute of Pathology (AFIP) Rawalpindi, spanning from June 2022 to May 2023. Ethical approval was obtained from the institutional ethical committee, and the study was

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conducted in compliance with the principles of the Helsinki Declaration, ensuring the rights and confidentiality of all participants. The sample size was calculated using Yamane's method, resulting in a total of 94 cases with a confidence level of 95% and a precision of 10% (12). The study included 53 cases of Classic Hodgkin Lymphoma (CHL) and 41 cases of Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL), all of which were diagnosed through either trucut biopsy or excision biopsy of the lymph node. Among the CHL cases, there were 32 cases of mixed cellularity subtype, 17 cases of nodular sclerosis subtype, and 2 cases of lymphocyte-depleted subtype. In 2 cases, no further subtyping was performed.

Tissue samples were fixed in 10% buffered formalin and processed using the TissueTek® tissue-processing equipment. The samples were subsequently embedded in paraffin wax, and 5 µm-thick sections were prepared using a semi-automated rotary microtome. These sections were mounted on glass slides and stained with conventional hematoxylin and eosin (H&E) dyes. Microscopic examination was performed to select cases with a confirmed diagnosis of CHL or NLPHL. Immunohistochemistry was carried out using the STAT6 antibody (clone EP325, Dako), following antigen retrieval using a citrate buffer protocol in a pressure cooker as the heating source. Sections from a diagnosed case of solitary fibrous tumor were utilized as external positive controls in each staining procedure. The expression of STAT6 in neoplastic cells was evaluated in both the nuclear and cytoplasmic compartments, with the staining patterns categorized as nuclear alone or combined nuclear and cytoplasmic.

Data on patients' demographic characteristics, including age, gender, and biopsy site, were systematically recorded. Statistical analysis was performed using SPSS software, version 25.0. Descriptive statistics were utilized to summarize the demographic and clinical characteristics of the study population, including frequency tables and percentages. Fisher's exact test was applied for inferential analysis to compare the expression patterns of STAT6 between CHL and NLPHL cases, with a significance level set at $p < 0.05$.

The study adhered to rigorous standards of data collection and analysis to ensure the validity and reliability of the findings. The thorough assessment of STAT6 expression patterns aimed to provide valuable insights into the differential diagnosis of Hodgkin lymphomas, with the potential to enhance diagnostic accuracy in clinical practice, particularly in settings with limited resources.

## RESULTS

A total of 94 cases of Hodgkin lymphoma were included in this study, comprising 53 cases of Classic Hodgkin Lymphoma (CHL) and 41 cases of Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL). Of the 94 patients, 65 were male (69.14%) and 29 were female (30.85%), with an age range of 3 to 69 years. Lymph node biopsies were obtained from various sites, including the axillary, cervical, inguinal, submandibular, and para-aortic regions. The intensity of STAT6 staining ranged from weak to strong, with the percentage of positive tumor cells varying between 5% and 70%.

Table 1 presents the nuclear expression of STAT6 in neoplastic cells. Nuclear positivity for STAT6 was observed in all cases of CHL (100%), with no negative cases. In contrast, nuclear expression of STAT6 was found in only 10 out of 41 cases of NLPHL (24.39%), with the remaining 31 cases (75.61%) showing no nuclear staining.

**Table 1: Nuclear Staining of STAT6 in Neoplastic Cells**

| Diagnosis | Positive | Negative | Total |
|---|---|---|---|
| Hodgkin Lymphoma (CHL) | 53/53 (100%) | 0/53 (0%) | 53 |
| Nodular Lymphocyte Predominant HL | 10/41 (24.39%) | 31/41 (75.61%) | 41 |

Table 2 displays the combined nuclear and/or cytoplasmic staining patterns of STAT6 in neoplastic cells. All CHL cases (100%) demonstrated both nuclear and cytoplasmic expression of STAT6. In contrast, none of the NLPHL cases exhibited combined nuclear and cytoplasmic expression; all cases showed either nuclear alone or cytoplasmic alone staining patterns.

**Table 2: Nuclear and/or Cytoplasmic Staining of STAT6 in Neoplastic Cells**

| Diagnosis | Positive | Negative | Total |
|---|---|---|---|
| Hodgkin Lymphoma (CHL) | 53/53 (100%) | 0/53 (0%) | 53 |
| Nodular Lymphocyte Predominant HL | 0/41 (0%) | 41/41 (100%) | 41 |

In summary, all cases of CHL exhibited strong nuclear and cytoplasmic STAT6 expression, confirming its utility as a diagnostic marker. In contrast, NLPHL cases were predominantly negative for nuclear STAT6 expression, which further supports the differentiation between CHL and NLPHL based on STAT6 staining patterns. The results underscore the diagnostic significance of STAT6, particularly its nuclear expression, in confirming a diagnosis of CHL and ruling out NLPHL, thus aiding in the accurate diagnosis and management of Hodgkin lymphomas.

## DISCUSSION

The findings of this study demonstrated the significant diagnostic utility of STAT6 expression in distinguishing Classic Hodgkin Lymphoma (CHL) from Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL).

STAT6, a key transcription factor in the IL-4/IL-13 signaling pathway, was shown to have a distinct nuclear expression pattern in CHL, with 100% of cases exhibiting nuclear positivity. This contrasts sharply with NLPHL, where nuclear expression was observed in only a small subset of cases, further highlighting the specificity of STAT6 as a marker for CHL. These results align with previous studies that have underscored the role of STAT6 in the pathogenesis of CHL and its potential as a diagnostic marker. For instance, Van Slambrouck et al. found a similar pattern of STAT6 expression, affirming its diagnostic relevance in distinguishing CHL from other lymphomas (15).

The study's strengths lie in its robust methodology, including the use of a well-defined patient cohort and standardized immunohistochemical techniques. The inclusion of a control group and the use of external positive controls for STAT6 staining provided additional rigor to the findings. Moreover, the statistical analysis was appropriately conducted, ensuring that the results are both reliable and applicable in clinical practice. However, there are several limitations to consider. The relatively small sample size, particularly in the NLPHL group, may limit the generalizability of the findings. Additionally, the study was conducted in a single institution, which may introduce selection bias and limit the applicability of the results to other populations. Future studies with larger, more diverse cohorts would be beneficial to further validate the diagnostic utility of STAT6 in Hodgkin lymphoma (8, 16).

Another limitation is the inherent variability in immunohistochemical staining, which can be influenced by factors such as tissue fixation, antigen retrieval, and antibody specificity. While the study attempted to control for these variables, some degree of variability is inevitable, and this could potentially affect the reproducibility of the results in other settings. The study also did not explore the potential prognostic implications of STAT6 expression in Hodgkin lymphoma, an area that warrants further investigation. Although STAT6 was shown to be a reliable marker for distinguishing CHL from NLPHL, its role in predicting patient outcomes or response to therapy remains unclear and should be a focus of future research (16).

The findings of this study contribute to the growing body of literature supporting the use of STAT6 as a diagnostic marker in Hodgkin lymphoma. The strong nuclear expression of STAT6 in CHL, in contrast to its limited expression in NLPHL, provides a valuable tool for pathologists in differentiating between these two entities, particularly in cases where traditional markers may be inconclusive. This is especially important in resource-limited settings where access to a comprehensive panel of immunohistochemical markers may be restricted. By incorporating STAT6 into the diagnostic workup, clinicians can enhance diagnostic accuracy and ensure that patients receive the most appropriate treatment.

## CONCLUSION,

In conclusion, this study confirmed the high diagnostic value of STAT6 nuclear expression in CHL, providing strong evidence for its incorporation into routine diagnostic protocols for Hodgkin lymphoma. Despite some limitations, the study's findings are robust and align with previous research, offering a reliable method for distinguishing CHL from NLPHL. Future research should aim to address the identified limitations and explore the potential prognostic significance of STAT6 in Hodgkin lymphoma, which could further enhance its clinical utility.

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