ROLE OF GATA3 AND STAT6 IMMUNOHISTOCHEMISTRY IN CLASSIC HODGKIN LYMPHOMA (CHL) AND NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA (NLPHL)

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ABSTRACT

Objective: The purpose of our study is to investigate the utility of immunohistochemical expression of GATA3 and STAT6 in differentiating Classic Hodgkin Lymphoma (CHL) from Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL).

Material and Methods: We approached database of Shaukat Khanum Memorial Cancer Hospital and Research Centre and selected a total of 79 CHLs and 15 NLPHLs diagnosed either on Trucut biopsy or excision biopsy diagnosed during the time 2020-2021. One slide from each of these 94 cases was stained with GATA3 and STAT6 independently and pattern of staining (either nuclear or cytoplasmic or dual nuclear and cytoplasmic) was assessed.

Results: 75/79(95%) cases of CHL were positive for GATA3 staining [60/79(75.9%) cases showed nuclear GATA3 staining, 15/79(18.9%) cases showed both nuclear and cytoplasmic staining] and 4/79(5%) CHL cases were negative for GATA3. 15/15(100%) cases of NLPHL were negative for GATA3[12/15(80%) cases showed no staining and 3/15(20%) cases showed only cytoplasmic blush]. 65/79(82%) cases of CHL were positive for STAT6[17/79(21.5%) cases showed nuclear STAT6 expression, 48/79(60.7%) cases showed both nuclear and cytoplasmic staining], and 14/79(18%) cases of CHL were negative for STAT6 expression [8/79(10%) cases showed only cytoplasmic staining and 6/79(8%) showed neither nuclear nor cytoplasmic staining]. 14/15(93.3%) cases of NLPHL were negative for STAT6[5/79(33.3%) cases showed only cytoplasmic staining and 9/15(60%) cases showed no staining] and 1/15(6.7%) case of NLPHL was positive for STAT6[showed nuclear STAT6 staining].

Conclusion: Nuclear staining of GATA3 and STAT6 either alone or in combination with cytoplasmic staining can be used to differentiate CHL from NLPHL. GATA3 effectively excludes NLPHL with 100% negative predictive value.

Keywords: GATA3, STAT6, Classic Hodgkin Lymphoma, Nodular Lymphocyte Predominant Hodgkin Lymphoma

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INTRODUCTION

Among the most common malignancy the Hodgkin lymphoma is ranked 6th globally. In Pakistan Hodgkin lymphoma comprises 4.9% of all reported malignancies (Samreen et.al 2019) [1]. The term Hodgkin lymphoma comprises two major types: Classic Hodgkin Lymphoma (CHL) and Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL). Both are having scattered large neoplastic cells in a rich reactive inflammatory milieu and arise from the germinal or post-germinal center, B cells. Patients are usually diagnosed in their early adulthood and lymphadenopathy with B symptoms is the most common presentation. However, CHL has a more aggressive course and is usually treated with chemotherapy. NLPHL has a more indolent biology and for patients with early-stage NLPHL without any B symptoms, Involve Site Radiation Therapy (ISRT) is a required treatment [2,3,4,5].

The neoplastic cells in NLPHL tend to maintain their B cell phenotype [6]. On the other hand, malignant cells of CHL (Reed-Sternberg Cells) have been shown an abnormal B cell phenotype. The most frequently used markers CD30, CD15 and PAX5 are usually positive in CHL. However, their expression is not uniform in all Reed Sternberg cells of the same patient. Some cases have even showed complete lack of staining for one of the markers. On the other hand, the LP cells are CD15 and CD30 negative and express CD20 and CD45 [7]. Although their diagnosis is straight forward in most cases, occasional cases pose diagnostic challenge. Hodgkin and Reed-Sternberg cells (HRS) produce several cytokines, creating their own reactive cell microenvironment, which may contribute to their growth and survival [8]. GATA3 is a T-cell Transcription factor partially involved through Nuclear Factor-kappa pathway as well as

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Notch-1 pathway and its aberrant expression has been seen in CHL through gene expression profiles (Kuppers *et al*) [9,10]. Cytokine signaling is also mediated through the Signal Transducer and Activator of Transcription (STAT) family of transcription factors. IL-4 and IL-13 also activate the STAT6. IL-13, IL-13R expressions are common features of HRS cells in HL [11,12,13,14]. This study we explored the utility of GATA3 and STAT6 immunohistochemical stains in differentiating CHL from NLPHL cases.

MATERIAL AND METHODS

The data is collected using two-stage sampling method. At first stage we selected Lahore District from all other districts of Punjab. In Lahore, there are many research centers of cancer like Shaukat Khanum Memorial Cancer Hospital and Research Centre, INMOL …etc.

At second stage, we selected the Shaukat Khanum Memorial Cancer Hospital and Research Centre. Now using the Yamane's method, with Confidence Level 95%, P<0.05, and 10% precision (Israel, 1992) we selected the sample size n=94 from the research Centre[15]. The sample is selected using Consecutive Sampling during the time 2020-2021.

After approval from the Shaukat Khanum Memorial Cancer Hospital and Research Centre's (SKMCH&RC) institutional Review Board (EX-06-10-20-01), the database of Shaukat Khanum Memorial cancer hospital and research Centre was used to select the sample of 79 CHLs and 15 NLPHLs diagnosed either on Trucut biopsy or excision biopsy. Among CHLs, 51 cases were of mixed cellularity subtype, 21 of nodular sclerosis type, 1 of lymphocyte depleted type and in 6 cases no further subtyping was done. All 94 cases were reviewed independently by two hematopathologists.

One slide per case was stained separately with GATA3 and STAT6 and pattern of staining (either nuclear or cytoplasmic or dual nuclear and cytoplasmic) was assessed. Isolated Nuclear expression or dual nuclear and cytoplasmic expression of GATA3 and STAT6 is required to consider the expression positive. For GATA3, cases of breast carcinoma served as external controls and for STAT6, cases of Solitary Fibrous Tumor served as an external control, however background small lymphocytes served as internal controls for both.

Clinical information including age, gender, site of lymph node and nature of biopsy were gathered. Descriptive analysis, frequency tables with percentages are evaluated for data representation. For inferential data analysis Fisher exact test using SPSS software is applied. The significance was set at <0.05.

RESULTS

A total of 65 male and 29 female patients were selected which were earlier diagnosed either on tru cut or excision biopsy. Age range varied from 3Y to 69Y.The lymph node site varied including axilla, cervical, mediastinal, inguinal, submental, sublingual, sub mandibular or para-aortic lymph nodes. Intensity of staining varied ranging from weak to strong positive. Percentage of positive tumor cells ranged from 5- 90%.

Table-I demonstrates GATA3 staining pattern in CHLs and NLPHLs. 75/79(95%) cases of CHL were positive for GATA3 staining [60/79(75.9%) cases showed nuclear staining, 15/79(18.9%) cases showed both nuclear and cytoplasmic staining]. The expression varied among different subtypes of CHL. All 51/51 cases (100%) of mixed cellularity subtype were positive (most consistent), 19/21 cases (90%) of nodular sclerosis subtype were positive, the only 1 case of lymphocyte depleted subtype was negative for GATA3 and 5/6 (83%) of CHL cases in which further subtyping was not done were positive. All [15/15(100%)] cases of NLPHL were negative for GATA3 [12/15(80%) cases showed no staining and 3/15(20%) cases showed only cytoplasmic blush (Figure-I). The *P* value was <0.001(Table-II)

Table-III demonstrates STAT6 staining pattern in CHLs and NLPHLs. 65/79 (82.2%) cases of CHL were positive for STAT6 [17/79(21.5%) cases showed nuclear STAT6 expression, 48/79 (60.7%) cases showed both nuclear and cytoplasmic staining]. The intensity of staining and percentage positive cells varied. Of the different subtypes of CHL, 40/51(78%) cases of mixed cellularity subtype were positive while 11/51 (22%) were negative, 20/21(95%) cases of nodular sclerosis subtype were positive (most consistent) and 1/21(5%) cases was negative, the only one case of lymphocyte depleted subtype was negative for STAT6 and of those CHL cases in which further subtyping was not done 5/6 (83%) cases showed positive staining and 1/6 (17%) case was negative. 14/15 (93%) cases of NLPHL were negative for STAT6 expression [9/15(60%) cases showed no staining and 5/15(33.3%) cases showed only cytoplasmic staining while only 1/15(7%) case was positive for STAT6 staining [nuclear expression] (Figure-I). The P value was <0.001 (Table-II)

Table-I: GATA3 Staining in Lymphomas *CHL NOS indicates those classic Hodgkin lymphoma cases in which further subtyping could not be done.

Entities	No of Positive cases/Total number of cases	% Positive cells	Nuclear staining	Nuclear and cytoplasmic staining	Cytoplasmic staining	Number of Staining
Classic Hodgkin Lymphoma	75/79	95	60/79 (75.9%)	15/79 (18.9%)	0/7(0%)	4/79 (5%)
Mixed cellularity variant	51/51	100	40/51 (78.4%)	11/51 (21.6%)	0/51 (0%)	0/51 (0%)
Nodular Sclerosis variant	19/21	90	15/21 (71.4%)	4/21 (19%)	0/21 (0%)	2/21 (9.5%)
Lymphocyte Depleted variant	0/1	0	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)
CHL NOS*	5/6	83	5/6 (83%)	0/6 (0%)	0/6 (0%)	1/6 (16.7%)
Nodular Lymphocyte Predominant Hodgkin Lymphoma	0/15	0	0/15 (0%)	0/15 (0%)	3/15 (20%)	12/15 (80%)

Table-II: Chi square test.

	Value	df	Asymp. Sig. (2 sided)	Exact Sig. (2 sided)
Pearson chi-Square	65.382*	12	0.000	<0.000
Likelihood Ratio	65.724	12	0.000	<0.000
Fischer's Exact Test	58.145			<0.000
N of valid cases	94			

Table-III: STAT6 staining in lymphomas.

Entities	Positive/ Total	% Positive	Nuclear staining	Nuclear and cytoplasmic staining	Cytoplasmic staining	Negative
Classic Hodgkin Lymphoma	65/79	82.2%	17/79 (21.5%)	48/79 (60.7%)	8/79 (10.1%)	6/79 (7.5%)
Mixed cellularity variant	40/51	78.4%	8/51 (15.7%)	32/51 (62.7%)	8/51 (15.7%)	3/51 (5.9%)
Nodular Sclerosis variant	20/21	95.3%	6/21 (28.6%)	14/21 (66.7%)	0/21 (0%)	1/21 (4.8%)
Lymphocyte Depleted variant	0/1	0%	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)
CHL NOS	5/6	83%	3/6 (50%)	2/6 (33.3%)	0/6 (0%)	1/6 (16.7%)
Nodular Lymphocyte Predominant Hodgkin Lymphoma	1/15	6.7%	1/15 (6.7%)	0/15 (0%)	5/15 (33.3%)	9/15 (60%)

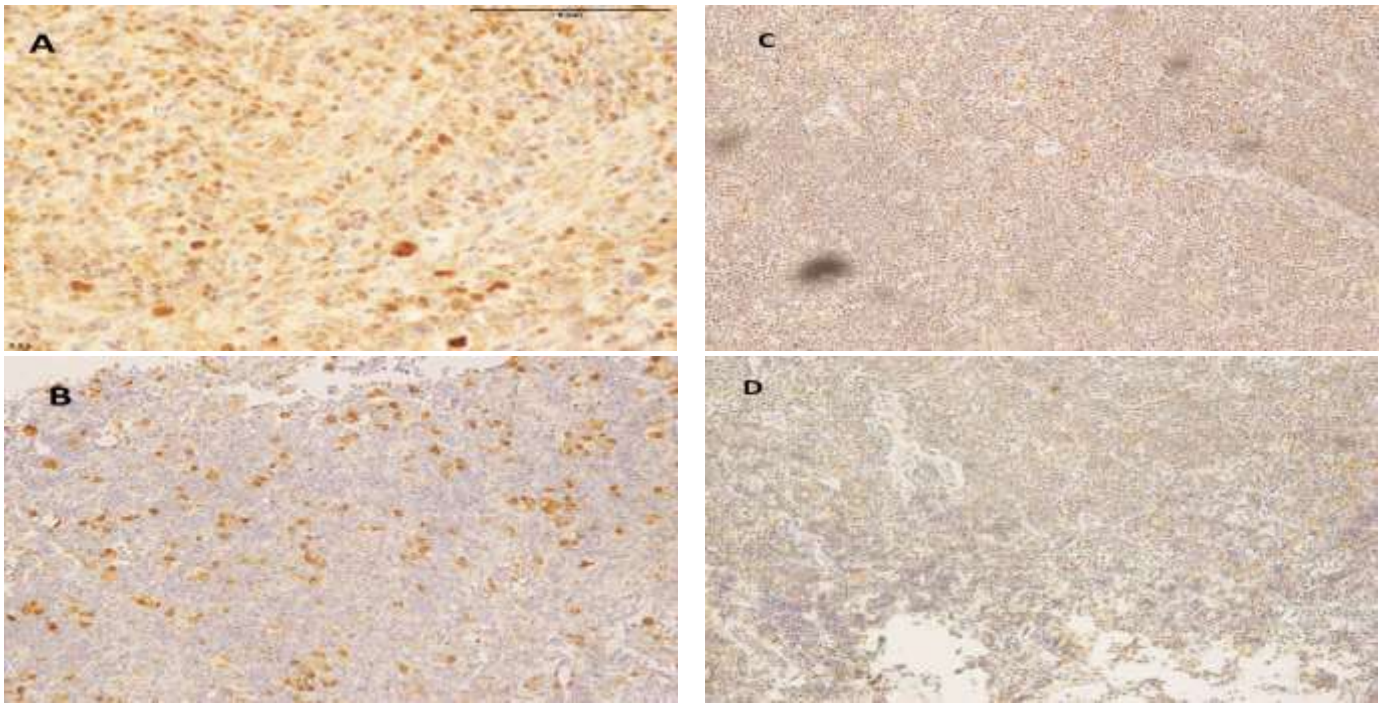

Figure-I: A, GATA3 staining in CHL. B, STAT6 staining in CHL. Images (A, B) demonstrate Reed Sternberg cells which are positive for GATA3 and STAT6 positive. C, GATA3 staining in NLPHL. D, STAT6 staining in NLPHL. Images (C, D) demonstrate LP cells which are negative for GATA3 and STAT6.

DISCUSSION

The distinct natural history, biology, prognosis and treatment modalities difference in between CHL and NLPHL obviates the need to accurately diagnose these two entities on tissue biopsy in both upfront and relapsed cases. The standard treatment guidelines for Classic Hodgkin Lymphoma patients is chemotherapy with vinblastine, decarbazine, bleomycin and doxorubicin [16]. GATA3 immunohistostain is a lineage specific stain for breast epithelium, urothelial and trophoblastic tumors and is available in most laboratories [17]. Similarly, STAT6, a member of STAT Transcription family, is a sensitive and specific stain for Solitary Fibrous Tumor and is widely available in laboratories. Nuclear expression of GATA3 and STAT6 is required to consider the expression positive. Our study aimed to utilize the expression of GATA3 and STAT6 in differentiating CHL and NLPHL.

Earlier studies have demonstrated nuclear expression of GATA3 in Reed Sternberg cells of CHL[18]. In a study conducted by Kezlerian *et al*. GATA3 nuclear expression was suggestive of CHL and ruled out NLPHL with 100% negative predictive value [17]. In our study, among CHL cases, nuclear expression was seen alone and also in combination with cytoplasmic staining. However, NLPHL cases either showed no expression of GATA3 or only cytoplasmic staining (considered negative) which effectively excluded NLPHL.

Similarly, previously conducted studies showed both nuclear alone and dual nuclear and cytoplasmic localization of STAT6 in classic Hodgkin lymphoma [19]. In the study conducted by Skinidder *et al*. the authors examined STAT6 nuclear expression in almost 80% of cases of CHL [12]. These results support our research with STAT6 showing positive nuclear expression restricted to classical Hodgkin lymphoma compared with nodular lymphocyte predominant Hodgkin lymphomas.

An important factor in both GATA3 and STAT6 expression is proper fixation of tissue. Both antibodies showed best performance in optimally fixed tissues and small biopsies, and showed poor sensitivity in suboptimally fixed areas of larger biopsies and in older biopsies. Therefore, the age and fixation status of tissue should be kept in account while using GATA3 and STAT6 immunohistochemical stains in the diagnostic pathology. In conclusion, nuclear expression of GATA3 and STAT6 is helpful in accurate distinction between Classic Hodgkin lymphoma and Nodular lymphocyte predominant Hodgkin lymphomas.

CONCLUSION

Nuclear staining of GATA3 and STAT6 either alone or in combination with cytoplasmic staining can be used to differentiate CHL from NLPHL. GATA3 effectively excludes NLPHL with 100% negative predictive value.

CONFLICT OF INTEREST

None

AUTHORS CONTRIBUTION

Nida Babar: Searched the data for the article and designed the whole manuscript.

Sajid Mushtaq, Muhammad Tariq Mehmood: Performed the histological review.

Asad Hayat Ahmed, Muddasar Hussain, Usman Hassan: Contributed to the discussion, final editing read and approved the final manuscript.

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