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Clinicopathological value of tumor-associated macrophages in adult classical Hodgkin lymphoma in Hawler city

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Abstract

Background and objective: The aims of the present research were to study the clinicopathological features of adult classical Hodgkin lymphoma (cHL) in Hawler city and to evaluate the significance of immunohistochemical expression of CD68 positive tumor associated macrophage in the tumor microenvironment as a marker for prediction of malignant progression.

Methods: This is a retrospective cross-sectional study carried out in Rizgary Teaching Hospital and Nanakaly Hospital in Hawler city –Iraq in which 110cases of cHL patients were enrolled from January 2015 to July 2022.Clinical data were obtained from the hospital's medical records. All specimens were revised for histopathological and immunohistochemical diagnosis of CD15 and CD30 followed by staining with CD68 using IHC.

Results: The IHC showed 56.36% of cases expressed intermediate staining score for CD68 immune marker whereas 27.27% expressed high staining score. A positive association was found between staging of the cases and CD68 immune expressions.

Conclusion: Positive CD68by immunohistochemical analysis on TAM have diagnostic significance, its overexpression may predict cancer progression.

Keywords: CD68; Classical Hodgkin's lymphoma (cHL); Tumor associated macrophage (TAM).

Introduction

It is well known that there are two major types of Hodgkin lymphoma (HL): classical HL (cHL), which currently account for 95% of all HL cases, and nodular lymphocyte predominant HL (NLPHL), HL is distinguished histologically by a scanty number of neoplastic cells known as Hodgkin and Reed/Sternberg (HRS) cells immersed in a rich inflammatory infiltrate of immune cells, including lymphocytes, macrophages, eosinophils, mast cells, plasma cells, and stromal cells, these cells participate to the microenvironment of the tumor.(1)

Generally, macrophages are essential cells in tumor microenvironments, they are typically described using the concept of functional polarization which identifies two types of macrophages, M1-polarized macrophages and M2-polarized macrophages (M1/M2 model) with distinct and opposite functions. It is commonly agreed that M1-polarized macrophages have anti-neoplastic impacts while M2 polarized macrophages are expected to accelerate tumorigenesis. M1 are classically activated macrophages that activated by T helper type 1 (Th-1) cells cytokines. M2 are alternatively activated macrophages develop in a T helper type 2 (Th-2) cells cytokine-rich microenvironments.(2,3)

The current research aims to explore the diverse clinicopathological parameters of classical Hodgkin's lymphoma (cHL) and to assess the value of CD68 immunohistochemical expressions on

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tumor associated macrophage (TAM) as predictors for disease progression.

Methods

In this study 110 FFPE blocks of cHL that had been surgically removed were collected from January 2015 to July 2022 from Rizgary Teaching Hospital and Nanakaly Hospital in Erbil – Iraq.

Specimen Collection and analysis

The ethical approval for handling human samples was attained from the Ethics Committee in Hawler Medical University. The handling of biological specimen was done according to the Declaration of Helsinki criteria.[4] From the Nanakaly hospital's database, clinical data including age, gender, B symptoms, bulky disease, stage, and bone marrow involvement were retrieved. The most relevant tissue block that was found to have the necessary elements was used to cut new sections, which were subsequently stained with haematoxylin and eosin (H&E) and histopathologically examined.

Immunohistochemistry:

The diagnosis of cHL made on the bases of H&E stains and positivity of tumor cell for CD15 and CD30 by IHC. In addition, a thin 4 µm section was taken for further analysis. Immunohistochemistry for CD68 was done using the avidin-biotin-peroxidase complex and primarily monoclonal antibodies raised against CD68, as well as the DakoCytomation EnVisionR+Dual link system-HRP(DAB+) staining protocol.

Tumor associated macrophages were determined as CD68+ cells using a monoclonal mouse antibody (Anti-human anti CD68 KPI, Dako). The percentage of CD68 positive macrophages was calculated in relation to the percentage of negative Hodgkin and Reed/Sternberg cells and reactive inflammatory cells in the background and was scored as I (5%), II (5-25%), and III (> 25%),[5-7] internal positive controls (endothelial cells lining adjacent vascular spaces) and negative controls (slides not incubated with primary antibodies) were used for quality control.

Statistical analysis:

Data entry, interpretation and graphing were performed using SPSS v22 and GraphPad Prism v8, The Chi-square was used to examine the relationship between the categorical variables. A *P* value of ≤ 0.05 was considered significant. *P* value of ≤ 0.01 were considered highly significant.

Results

This study included a total of 110 cases of cHL. Sixty one point eight percentage, (61.8%) were male, while 38.2% were females. Age of the studied cases were grouped as young, (patients age 15-34 years), which were 52.7% of the studied cases, young-adult, (patients age 35-50 years), which were 39.1% of the studied cases and elderly, (patients more than 50 years), which were 8.2% of the studied cases. Age of the cases ranged between 15-82 years with mean of 34.77years

Histopathological diagnosis revised and histologically the cases were divided as nodular sclerosis, 62.7% of the studied cases, (Figure 1A), mixed cellularity, 30.9% of the studied cases and others, (include lymphocyte rich, lymphocyte depleted and unclassified), that were 6.4% of the studied cases.[11] Staging of the studied cases were divided into four subtypes, stage one (stage I),10.9% of the studied cases, stage two (stage II), 52.7% of the studied cases, stage three (stage III), 20% of the studied cases and stage four (stage IV),16.4% of the studied cases.[8]

Fifty-one point eight, (51.8%) of the studied cases presented with B symptoms at time of diagnosis, while 48.2% of the studied cases were free of B symptoms at original time of the disease diagnosis. Seven point three, (7.3%) of the studied cases had involvement of bone marrow by the disease process at diagnosis, opposite to 92.7% of the studied cases that their bone marrow was tumour free at diagnosis. According to the radiological investigation,

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11.8% of the studied cases had Bulky disease at diagnosis, while 88.2% of the studied cases don't have bulky disease at diagnosis. Table 1 shows the number and percentages of the studied cases.

Table 1 Clinicopathologic characteristics of cases

Variables	Categories	No.	Percentage (%)
Age	Young	58	52.7
	Youngadult	43	39.1
	Elderly	9	8.2
Gender	Male	68	61.8
	Female	42	38.2
Stage	I	12	10.9
	II	58	52.7
	III	22	20
	IV	18	16.4
B symptoms	Present	57	51.8
	Absent	53	48.2
BM involvement	Involved	8	7.3
	Not involved	102	92.7
Histological type	Nodular Sclerosis	69	62.7
	Mixed Cellularity	34	30.9
	Others	7	6.4
Bulky Disease	Yes	13	11.8
Total		110	100.0

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CD68 immunohistochemical staining gave the following results: low, (score I), (< 5% of macrophages were stained), that were detected in 16.36% of the studied cases, intermediate, (score II), (5–25% of macrophages were stained), that were detected in 56.36% of the studied cases and high (score III), (> 25% of macrophages were stained) that were detected in 27.27% of the studied cases, (Figure 1B and Figure 2).

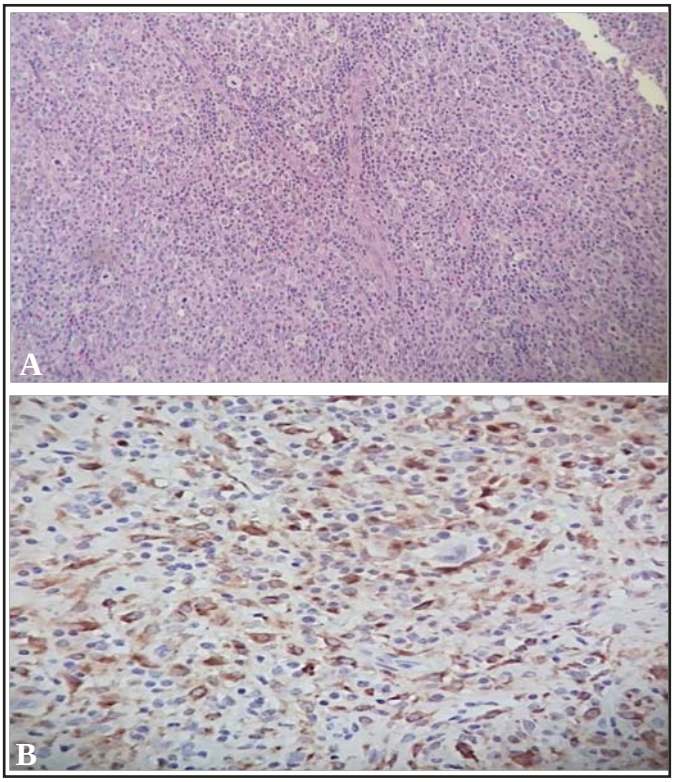

Figure 1 classical Hodgkin lymphoma, A, H&E stain. B, Immunohistochemical expression of CD68 on macrophages

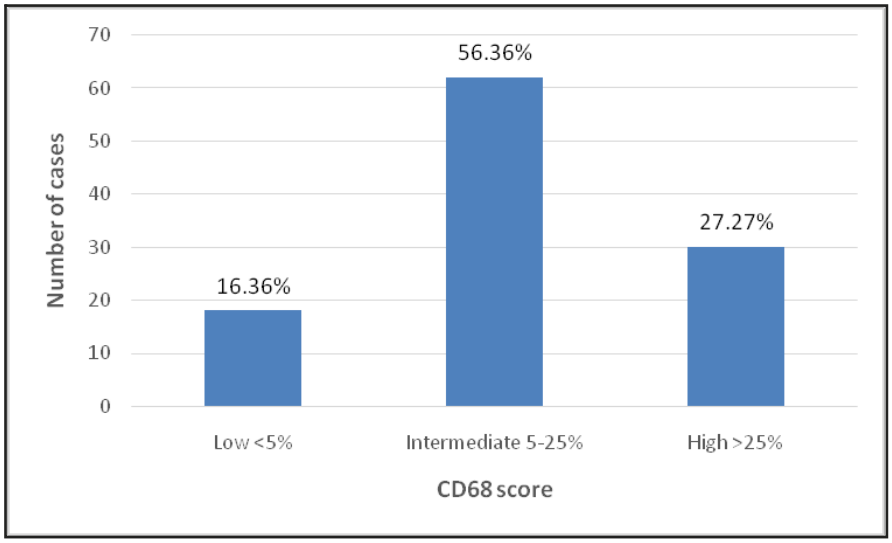

Figure 2 Number and percentages of cases according to CD68 score

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There was no statistically significant correlation between CD68 expression and patient age, gender, bulky disease, bone marrow involvement, B symptoms and histological type whereas a significant relation achieved with disease staging with *P* value = 0.011. (Table 2).

Table 2 Association between some clinicopathologic variables and CD68 immunoreactivity

Variables	Categories (No.)	CD68 No. (%)			*P* value
		Low	Intermediate	High	
Age	Young (58)	9 (15.5%)	29(50%)	20 (34.5%)	0.456
	Young adult (43)	8 (18.6%)	27 (66%)	8 (18.6%)	
	Elderly (9)	1 (11.1%)	6 (66.7%)	2 (22.2%)	
Gender	Male (68)	12 (17.6%)	38 (55.9%)	18 (26.5%)	0.892
	Female (42)	6 (14.3%)	24 (57.1%)	12 (28.6%)	
Stage	I (12)	4 (33.3%)	5 (41.7%)	3 (25%)	0.011
	II (58)	8 (13.8%)	41 (70.7%)	9 (15.5%)	
	III (22)	2 (9.1%)	11 (50%)	9 (41%)	
	IV (18)	4 (22.2%)	5 (27.8%)	9 (50%)	
B symptoms	Present (57)	9 (15.8%)	30 (52.6%)	18 (31.6%)	0.571
	Absent (53)	9 (17%)	32 (60.4%)	12 (22.6%)	
BM involvement	Involve (8)	1 (12.5%)	3 (37.5%)	4 (50%)	0.323
	Not involve (102)	17 (16.7%)	59 (57.8%)	26 (25.5%)	
Histological type	Nodular sclerosis (69)	9 (13%)	42 (60.9%)	18 (26.1%)	0.682
	Mixed cellularity (34)	7 (20.6%)	17 (50%)	10 (29.4%)	
	Others (7)	2 (28.6%)	3 (42.9%)	2 (28.6%)	
Bulky disease	Yes (13)	4 (30.8%)	5 (38.5%)	4 (30.8%)	0.249
	No (97)	14 (14.4%)	57 (58.8%)	26(26.8%)	

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Discussion

Many recent studies have focused on the impact of non-neoplastic cells on disease pathobiology, specifically immunohistochemical typing of cells in the microenvironment, with the goal of identifying potential prognostic markers and therapeutic targets in adult cHL. A unique feature of HL is that the neoplastic cells crucially depend on the supporting microenvironment and its cellular composition, particularly TAM. However, the role of TAM in adult classical Hodgkin lymphoma (cHL) patients remains controversial, and there is a great debate in the literature about its prognostic and diagnostic significance. Compared to other lymphomas, the cellular background infiltration is more intimately associated with the lymphomagenesis of cHL, histiocytes and CD4+ cells are drawn in and become activated as a result of the numerous cytokines and chemokines that HRS cells produce, in turn, HRS cells react to the chemokines and growth factors that these neighboring cells produce, and these latter substances act as vital feedback signals to promote proliferation and prevent apoptosis in HRS cells.[9,10]

Concerning the clinicopathological outcomes of the current study, it showed that male made up 61.8% of the studied cases, which is in agreement with most other studies that showed male gender predilection,[6,11] whereas few studies showed the reverse finding[5,12] that may be due to racial and geographical factors and population differences.

In terms of age, patients ranged from 15 to 82 years old, with a mean of 34.77; this finding is consistent with a previous study conducted in the same center of the current study by Lilan et al. in Erbil, Iraq, in 2019 [13] and many other studies.[5,6,11,12] Cases age less than 14 years were excluded from the study as childhood cHL may have different pathogenesis.[14]

Many previous studies reported that most cases were presented without bone marrow involvement by the disease process, this is nearly similar to the results of the current study.[5,6,11,12]

Regarding histological type, nodular sclerosis is the most frequent type; it constitutes 62.7% of the total studied cases. Mixed cellularity type follows as the second most common; it constitutes 30.9% of the total involvedcases,[6,11,12] we considered all other histological subtypes together because of small number of cases in each categories, so considering them together make the statistical analysis more reliable. A study done by Mona *et al* in Egypt showed slightly more common mixed cellularity type over nodular sclerosis,[5] these minor discrepancy may be as a result of difference in sample size and case selection bias.

As for B symptoms at presentation, findings in this study showed slightly more than half of cases were presented with B symptoms (51.8%), this result is in agreement with other studies,[5,7] while another study showed that most of cases of cHL were free of B symptoms at diagnosis.[11] A study done by Lilan *et al* in same center of this study, (Nanakaly center, Erbil/ Iraq) from the period of 2012-2016, also showed larger number of cases presented with B symptoms, (67.2%),[13] these mild improvement in presentation may indicate better early diagnostic strategies.

Bulky disease was present only in 11.8% of the studied cases, a finding that is similar to nearly all recent studies done.[5,6,11,12]

This study found that most of the cHL cases in this locality were presented with stage II disease (52%), followed by stage III and stage IV, while the least presentation by stage is stage I, consisting of 10.9% of the studied cases, findings that were nearly similar to most others.[5,6,11,12]

Since macrophages are heterogeneous cells that play an important role in altering the tumor immune microenvironment and have been identified as a pan-macrophage biomarker by CD68, the relationship between TAM and the outcome of various

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malignancies has caught the attention of many researchers.[2,10,16,17] In the assessment of relationships between CD68 immunohistochemical expressions and studied clinicopathologic features, the current study reached to a significant correlation between disease stage and CD68 protein expression, Osama *et al.* and Mona *et al.* from Egypt and other recent studies had the same findings; however, Mona et al. reached a significant association with bulky disease in addition.[5,6,11,12]

In the process of tumorigenesis, macrophages evolve, resulting in the properties of TAMs that promote tumor growth. Evidence suggests that secretions or exosomes from tumor cells shift the transcriptional program of TAMs from the M1-like phenotype to the M2-like phenotype. In a variety of cancers, the infiltration of M2 TAMs is significantly related to poor prognosis, tumor progression, and other adverse clinical outcomes.[18]

Many more clinical trials are being conducted to validate the relationship between macrophage infiltration or phenotype and the outcomes of patients receiving anti-immune therapy. Actually, it is important to specify macrophage subpopulations that have the potential to benefit from different targeted therapies; the difficulty comes from the dynamic nature of TME and the influences of other external factors.

Conclusion

Increased expression of CD68 in TAM as assessed by immunohistochemical method in adult cHL was associated with advanced tumor stage, however, no relation was identified with other studied clinicopathological features including age, gender, histological type, bulky disease, BM involvement and B symptoms at disease presentation.

Competing interests

The authors declare that they have no competing interests.

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