

The value of B-cell lymphoma 2 and CD20 markers for predicting response to treatment and disease recurrence in patients suffering Hodgkin's disease

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Abstract

Introduction: The specific markers on the cell surface can facilitate early diagnosis of the disease and also predicting response to treatment, disease recurrence and even patients' survival in cancer patients.

Objectives: The present study aimed to assess the two specific markers of Bcl-2 (B-cell lymphoma 2) and CD20 for predicting lack of response to treatment, and disease recurrence in patients suffering from Hodgkin's disease.

Patients and Methods: This case-control study was conducted on patients with classical Hodgkin's lymphoma, who are resistant to treatment with relapse (case group; n=31) and gender and age-matched patients' group appropriately responder to the conventional treatment (control group; n = 31). Two slides of specimens in each group were stained with the H&E stain and two other sections with monoclonal antibodies Bcl-2 and CD20.

Results: The positivity of CD20 and Bcl-2, markers was overall 21% and 27.4% respectively. The expression of Bcl-2 increased the risk of resistance to treatment or relapse by 2.3 times. CD20 expression reduced the risk of treatment resistance or relapse by 4.4 times. Based on the multivariable logistic regression model, the expression of Bcl-2 (OR = 7.300, $P=0.026$) was revealed as a marker of increasing resistance to treatment or the risk of relapse. While, expression of two CD20 markers (OR = 0.92, $P=0.016$) was recognized as a marker of reducing or resistance to treatment or recurrence risk of disease.

Conclusion: Increasing the expression of Bcl-2 marker and decreasing the expression of CD20 marker are considered as determinant factors associated with recurrence of disease or lack of response to treatment in classical Hodgkin's lymphoma.

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Introduction

Hodgkin's disease is a potentially treatable lymphoma with a distinct clinical, histological and biological profile. The disease is characterized by microscopic manifestations and histology as well as by surficial cell markers (1). Therefore, for diagnosis of the disease, histological evaluation is absolutely necessary and biopsy of the lymph nodes is required. The disease stage can be also characterized on medical history, clinical manifestations, and imaging (2). There are some unusual features in Hodgkin's disease that help to diagnose the disease such as the axial distribution in the lymph nodes, the presence of a large number of completely normal cells and eosinophils with malignant cells (3-5). Lymphoid hyperplasia is common, often before or with the onset of the disease. Despite these

Core tip

The specific markers on the cell surface can facilitate early diagnosis of the disease and also predicting response to treatment, disease recurrence and even patients' survival in cancer patients. Increasing the expression of B-cell lymphoma 2 marker and decreasing the expression of CD20 marker are considered as determinant factors associated with recurrence of disease or lack of response to treatment in classical Hodgkin's lymphoma.

clinical and laboratory features, in a number of patients, the differentiation of Hodgkin's lymphoma is simply not possible from other diseases of the lymphatic system, such as anaplastic large cell lymphoma. Thus detection of some specific cellular markers should be applied (6-8). Not only these markers can facilitate early diagnosis of the disease, but also may be beneficial in



predicting response to treatment, disease recurrence and even patients' survival (9).

Objectives

The present study aimed to assess the two specific markers of Bcl-2 (B-cell lymphoma 2) and CD20 for predicting resistance to treatment, and disease recurrence in patients suffering from Hodgkin's disease.

Patients and Methods

Study population

This case-control study was performed on patients with classical Hodgkin's lymphoma, who are resistant to treatment or disease relapse (case group) and gender and age-matched patients' group appropriately responder to the conventional treatment (control group). Both groups are from Firoozgar and Rasoul-e-Akram hospitals in Tehran. Initially, the hematoxylin and eosin (H&E) and immunohistochemistry (IHC) slides were re-examined to confirm the diagnosis of Hodgkin's lymphoma. Then, H&E slides and paraffin blocks were selected, especially the high-density areas of Reed–Sternberg cells. After selecting the best and most suitable paraffin block, four sections with a thickness of 4 microns were cut from the cross section of each specimen by microtome cutting and then deparaffinized. Two slices were stained with H&E technique and two other sections stained with monoclonal antibodies of Bcl-2 (Biogenex) and CD20 (Dako) based on commercial kits. It should be noted that for both antibodies, the coloring of at least 10% of tumor cells was considered as positive.

Ethical issues

Human rights were respected in accordance with the Helsinki Declaration 1975, as revised in 1983. The study was approved by ethics committee of Iran University of Medical Science. Human rights were respected in accordance with the Helsinki Declaration 1975, as revised in 1983. This study was conducted on pathology specimens. The study was conducted as a residential thesis of Seyed Ali Mohammadi in Pathology Department, Iran University of Medical Sciences, Tehran, Iran (Ethical code # IR.IUMS.REC.1394.9211100008).

Statistical analysis

For statistical analysis, results were presented as mean \pm standard deviation (SD). For quantitative variables and were summarized by absolute frequencies and percentages for categorical variables. Normality of the data was analyzed using the Kolmogorov–Smirnov test. Categorical variables were compared using chi-square test or Fisher's exact test when more than 20% of the cells with expected count of less than 5 were observed. The quantitative variables were also compared with t test or Mann-Whitney U test. For the statistical analysis, the statistical software SPSS version 16.0 for Windows (SPSS Inc., Chicago,

IL) was used. Accordingly, *P* values of 0.05 or less were considered statistically significant.

Results

A total of 62 patients (31 patients in the group with treatment resistance or relapse and 31 patients with classic Hodgkin's lymphoma who responded to treatment without relapse) were enrolled. There was no significant difference in mean age (36.30 ± 15.70 years versus 32.00 ± 14.10 years, *P* = 0.265) and male gender (64.5% versus 61.3%, *P* = 0.662) across the two groups (Table 1). In terms of the frequency of CD20 expression, in general, positive cases for CD20 were 12.0% that was significantly lower in the case group with treatment resistance and relapse when compared to the control group (9.7% versus 32.3%, *P* = 0.025). Furthermore, CD20 expression reduced the risk of treatment resistance or relapse by 4.4 times.

Generally, 27.4% of our cases demonstrated Bcl2 expression. On the other hand, 38.7% of refractory/relapse cases and 16.1% of control cases were positive for Bcl2 expression, indicating a significant difference (*P* = 0.046). In this regard, expression of Bcl-2 increased the risk of resistance to treatment or relapse by 2.3 times. Moreover, 54.8% of our cases express CD15 while expression in refractory/relapse cases and control cases were 35.5% and 74.2% respectively, that showed a significant difference (*P* = 0.002). The expression of CD15 reduced the risk of drug resistance or relapse by 2.5 fold. Based on the multivariable logistic regression model (Table 2), the expression of Bcl-2 (OR = 7.300, *P* = 0.026) was revealed as a marker of increasing resistance to treatment or the risk of relapse. Accordingly the expression of two CD20 markers (OR = 0.92, *P* = 0.016) and CD15 (OR = 0.41, *P* = 0.005) was recognized as a marker of reducing treatment resistance or recurrence risk. Accordingly, age and gender were not recognized as factors influencing the resistance to treatment or the risk of recurrence.

Discussion

The prediction of the outcome of patients with classic Hodgkin's lymphoma is always clinically important while increasing the stage of the disease can worsen the prognosis. Additionally, there are various criteria for predicting the prognosis of the disease, such as age, stage of the disease, or type of tumor. However, none of the pointed

Table 1. Baseline characteristics of the study population in case and control groups

Item	Case group	Control group	<i>P</i> value
Gender			0.265
Male	20 (64.5%)	19 (61.3%)	
Female	11 (35.5%)	12 (38.7%)	
Mean age, year	36.30 \pm 15.70	32.00 \pm 14.10	0.662
CD20 positivity	3 (9.7%)	10 (32.3%)	0.025
Bcl-2 positivity	12 (38.7%)	5 (16.1%)	0.046

Table 2. Multi-variable logistic regression model to determine the role of cell markers to predict response to treatment and disease relapse

Item	B	SE	Wald	P value	Exp(B)	95% CI for EXP(B)	
						Lower	Upper
Age	-0.009	0.024	0.133	0.715	0.991	0.947	1.038
Sex	0.367	0.704	0.272	0.602	1.444	0.363	5.743
CD20	-2.385	0.993	5.771	0.016	0.092	0.013	0.645
Bcl-2	1.988	0.896	4.926	0.026	7.300	1.262	42.234
CD15	-1.931	0.688	7.878	0.005	0.145	0.038	0.558
Type			2.217	0.330			
Type (1)	-0.796	1.171	0.462	0.496	0.451	0.045	4.476
Type (2)	-1.446	1.012	2.040	0.153	0.236	0.032	1.713
Constant	3.739	2.313	2.614	0.106	42.069		

indicators have been able to predict disease prognosis with high precision. Therefore, the use of laboratory diagnostic markers with high accuracy can be helpful in determining the outcomes of the disease (in particular, the prediction of response to treatment and the risk of recurrence). Various surface cell markers such as CD20 and Bcl-2 have recently been evaluated. In addition, to determine their frequency and specificity as surface markers of lymphatic cells, their role in predicting the outcomes of the disease has been studied. In this study, the first step was to determine the frequency of the expression of each of the pointed markers in patients with classical Hodgkin's lymphoma, and in the second stage, the role of each one was tested for predicting resistance to treatment or relapse.

In our study, the frequency of CD20, Bcl-2, and CD15 markers was 21%, 27.4% and 54.8% respectively, which was completely within the range defined in other studies. Additionally, in assessing the diagnostic value of markers in predicting poor prognosis, we showed that expression of Bcl-2 was recognized as a factor increasing resistance to treatment or the risk of recurrence while the expression of two markers of CD20 and CD15 regarded as reducing treatment resistance or the risk of recurrence. Since CD15 staining has different results depending on the type of clone, the statistical results of this marker are not reliable. In fact, only two CD20 and Bcl-2 markers have high potential for predicting the disease recurrence and can be exploited along with other predictive factors such as the stage of the disease. In other words, in prognostic systems related to prognosis and recurrence of the disease, the expression of Bcl-2 and the reduction of the expression of the CD20 marker are considered as a factor associated with relapse of the disease or failure to respond to treatment in the classic Hodgkin's lymphoma.

In other studies, we have had similar results. In the study of Barakzai et al (10), CD20 marker was reported in 19.8% of patients. In the study of Portlock et al (11), the prevalence of CD20 expression was 11% within the 29-month follow up of the patients, CD20 reduction was a predictor of relapse. In the study by Rassidakis et al (12), CD20 positivity was confirmed in 22% of patients. The 5-year survival rate for patients with and without CD20 expression was 86% and 84% respectively, indicating an increase in survival

in patients with positive CD20 expression. In the study by Tzankov et al (13), the expression of CD20 marker was confirmed in 20% of patients. During a 12-year follow up, the increase in recurrence was associated with a reduction in the expression of CD20 (negative CD20). In fact, rates of recurrence in the CD20 (+) and CD20 (-) groups were 8% and 32%, respectively.

Concerning the association between Bcl-2 expression and disease prognosis, the results were similar. In the study by Bai et al (14), Bcl-2 positivity was reported in 27% of patients. In the study by Sup and colleagues (15), Bcl-2 was expressed in 26% of patients that was associated with lower survival rates. In the multivariate regression model, the factors associated with disease prognosis were age over 45, stage III to IV, and expression of Bcl-2. In the study by Rassidakis et al (12), the 5-year survival of patients was 74% for cases with expression of Bcl-2 versus 84% for cases without expression of this marker.

Conclusion

As a general conclusion, in our study, the frequency of CD20 and Bcl-2 markers in classical Hodgkin's lymphoma was 21%, 27.4% and 54.8% respectively, which was completely within the range defined in other studies. In addition, increasing the expression of Bcl-2 marker and decreasing the expression of CD20 marker are considered as determinant factors associated with recurrence of disease or lack of response to treatment in classical Hodgkin's lymphoma.

Study limitations

This study was conducted on a limited proportion of specimens and should re-examined with larger samples

Authors' contribution

SAM designed the study, observed accuracy and validity of the study. AZM and AB collected the data and follow the study. SAM supervised the project. SAM wrote the paper. All authors edited and revised the final manuscript and accepted its publication.

Conflicts of interest

The authors declare no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication,

falsification, double publication or submission, redundancy) have been completely observed by the authors.

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