

Intercellular Adhesion Molecule-1 (ICAM-1), CD44s Expression, and Serum Level of sICAM-1 in Disseminated non-Hodgkin's Lymphoma: Correlation with Overall Survival

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ABSTRACT

Background and Purpose: Uncontrolled growth, invasion and distant spread are the characteristics of neoplastic cells. Changes in adhesion molecule expression, such as loss of expression, de novo expression or functional alterations, can be observed in each of these steps. The aim of the present work was to study the expression of two adhesion molecules [standard CD44 (CD44s) and intercellular adhesion molecule-1 (ICAM-1)] and the soluble form of ICAM-1 (sICAM-1) in adult disseminated non-Hodgkin's lymphoma (D-NHL) and to correlate the findings with overall survival.

Patients and Methods: The expression of CD44s and ICAM-1 adhesion molecules and the level of sICAM-1 were studied in 34 cases of (D-NHL). They included: 13 cases of lymphoblastic lymphoma (LBL), 5 cases of Burkitt's lymphoma (BL), one case of diffuse large cell lymphoma (DLCL), 14 cases of small lymphocytic lymphoma (SLL), one case of mantle cell lymphoma (MCL). Ten apparently healthy individuals were taken as a control group. CD44s expression was evaluated by direct immunofluorescence, ICAM-1 by immunoperoxidase on cytopreps and serum level of sICAM-1 by ELISA.

Results: ICAM-1 was positive in 6/34 cases (17.6%) of D-NHL. ICAM-1 was expressed in 2/15 (13.3%) of low-grade NHL and 4/19 (21%) of high-grade lymphoma with no significant difference between the two groups ($p=0.6$). CD44s was expressed in 13/34 cases (38.2%) of D-NHL; in 4/15 (26.6%) of low grade NHL and 9/19 (47.3%) of high grade lymphoma with no significant difference between the two groups ($p=0.2$). The serum levels of sICAM-1 were elevated in all patients with D-NHL compared to healthy controls with a statistically significant difference ($p<0.001$), (median 1160 ng/ml, range from 150 to 2500 ng/ml, and 375 ng/ml, range from 270 to 620 ng/ml, respectively). The median of sICAM-1 in patients with high-grade D-NHL was significantly ($p=0.006$) lower than that of those with low-grade D-NHL (median 890 ng/ml, range from 150 to 1540 ng/ml and 1440 ng/ml, range from 380 to 2500 ng/ml respectively).

The median follow-up duration for all patients was 18 months. No statistical significant difference was achieved ($p=0.8$) on comparing overall survival pattern between D-NHL lymphoma patients with positive or negative expression of ICAM-1 or sCD44 expression. Also, no statistically significant difference ($p=0.9$) was found between patients with sICAM (above and below 600 ng/ml) at 18 months from diagnosis.

Conclusion: There is a marked heterogeneity in cell adhesion molecule (CAM) expression in NHL and this may correlate with degree of differentiation of malignant lymphocytes. However the exact significance of these findings will require functional studies to determine the role of these CAMs in each subtype of NHL.

Key Words: Disseminated non-hodgkin's lymphoma - ICAM-1 - CD44s - sICAM-1 - Overall survival.

INTRODUCTION

Adhesion molecules are transmembranal cell surface proteins that allow cells to communicate with each other or with the extracellular matrix. These molecules are involved in physiologic processes such as embryogenesis, inflammatory response, immunosurveillance, cell growth and repair, hematopoiesis and hemostasis. Uncontrolled growth, invasion and distant spread are characteristic of neoplastic cells. Changes in adhesion molecule expression, such as loss of expression, de novo expression or functional alterations, can be observed in each step [1].

Adhesion molecules play a critical role in lymphopoiesis by regulating the interaction between lymphocyte precursors and stromal

cells in the bone marrow microenvironment. Lymphocytes circulate throughout the body by traveling through the blood stream, moving into tissues and returning to the major circulation via lymphatic system. Adhesion molecules on lymphocytes and their counterparts on endothelial cells determine the specificity of lymphocyte recirculation and homing. ICAM-1 is an immunoglobulin-like molecule with important functions in the immune activation and immune response requiring cell-cell contact. Most of the biologic effects of ICAM-1 occur through its interaction with the β 2-integrin named lymphocyte function antigen-1 (LFA-1) on the opposing cell. sICAM-1 is the soluble form of ICAM-1. CD44 is a homing receptor that allows lymphocyte to bind to high endothelial venules [2].

Non-Hodgkin's lymphoma results from malignant proliferation of cells of lymphohistiocytic lineage. Any change in the mechanisms regulating normal lymphocyte traffic and homing can be considered to be responsible for the clinical course of the disease [3]. Therefore, the aim of the present work is to study the expression of two adhesion molecules (CD44s and ICAM-1) and the serum level of sICAM-1 in adult patients with D-NHL and to correlate the findings with patients overall survival at 18 months follow-up from diagnosis.

PATIENTS AND METHODS

Thirty four patients diagnosed as D-NHL were included in the study between August 2002 and July 2003. The diagnosis of D-NHL was based on: Morphologic examination of lymph node biopsy, bone marrow aspirate containing more than or equal 30% lymphoma cells, immunophenotyping, clinical examination, imaging, and complete blood picture. Patients whose bone marrow aspirate contained less than 30% lymphoma cells or were in stage below IV, those who did not have lymph node biopsies and those with small sample size of bone marrow aspirate were excluded from the study. After proper diagnosis patients were followed for a median of 18 months. Recruited patients were adults who presented to the outpatient clinic of National Cancer Institute, Cairo University. Ten healthy individuals (mostly were colleagues and employees in NCI) volunteered as a control group. Histopathologic classification of patients was done according to the World

Health Organization (WHO) classification of hematologic malignancy.

1- Preparation of mononuclear cell suspension from bone marrow aspirate for immunoperoxidase and direct immunofluorescence staining was done according to Peper et al. [4]:

I- *Immunoperoxidase staining*: All cases were investigated with a panel of specific B-cell antigens [CD19, CD20, CD22, cytoplasmic μ (polyclonal), κ (polyclonal), λ (polyclonal)], B-cell associated antigens: CD10, and CD23; T-cell specific antigens (CD1, CD2, CD3, CD7, CD4, CD8); T-cell associated antigens: CD5; stem cell antigen CD34, non-lineage associated antigen HLA-DR, and the adhesion molecule ICAM-1 (CD54). All monoclonals were produced in mice except (μ , κ , λ) were produced in rabbit. All monoclonals were obtained from Dako laboratories except CD54 was obtained from Novocastra, United Kingdom (cat#CP53). Positive staining was defined as a distinct dark brown ring at the periphery of the cells. 5% or more positive cells were considered as a positive score for the tested molecule [5].

II- *Direct immunofluorescence staining*: CD44s-FITC monoclonal antibody was diluted 1:5, then one drop was applied to the previously fixed cytopreparations, incubated in humid chamber for one hour, slides were then washed with PBS, mounted and examined under a fluorescence microscope [6].

2- *Serum ICAM-1*: The sICAM-1 levels in serum were determined by enzyme linked immunosorbent assay (ELISA) kits purchased from Beckman Coulter Company, U.S.A.

Statistical methods: Statistical package for social science (SPSS) version 10 was used for data base construction, management and analysis. Data were revised twice for quality assurance. Quantitative data were summarized in the form of means, standard deviation, median, minimum, and maximum. Qualitative data were summarized using frequencies and percentages. Fisher exact test was used to test the assessment of qualitative variables. Kruskal-Wallis test was used to detect the difference between serum ICAM-1 (ng/ml) levels in low-grade, high-grade and control groups. Mann-Whitney test was used to compare the level of serum ICAM-1 in patients and control. Overall survival for patients was assessed using the Kaplan Meier method.

Comparisons of survival pattern for all studied variables were done using the log-rank test. *p*-value was considered significant when ≤ 0.05 [7].

RESULTS

The present study was conducted on 34 newly diagnosed patients of D-NHL. The clinical, histopathologic, adhesion molecule expression and the level of sICAM-1 in patients are listed in table (1). Low-grade lymphomas were 14 SLL, and one MCL. High-grade lymphomas were 13 LBL, 5 BL, and one DLCL.

ICAM-1 expression: ICAM-1 was positive in 1/14 of SLL (n=14), and positive in MCL (n=1). ICAM-1 was expressed in 3/4 common B-cell, 1/4 pre-B-cell, and it was negative in one pro-B (n=1) as well as all T-cell LBL (early-T) or mediastinal lymphoma (n=4). In addition, it was negative in all cases of BL (n=5) as well as in DLBCL (n=1). The difference detected when ICAM-1 expression was compared between high and low-grade NHL was not significant ($p=0.672$). ICAM-1 was not expressed in controls Table (2).

CD44s expression: CD44s was positive in 3/14 of SLL, in MCL (n=1), 6/13 LBL and 3/5 BL. The difference of CD44s expression between high and low-grade NHL was not significant ($p=0.296$). CD44s was not expressed in controls Table (2).

Serum ICAM-1: The median level of sICAM-1 was 1160 ng/ml, ranging from 150ng/ml to 2500 ng/ml in D-NHL patients, and it was 375 ng/ml, with a range from 270 ng/ml in controls and the difference was statistically significant ($p<0.001$). When compared in high, low-grade lymphomas, and controls the level of serum ICAM-1 showed a significant difference ($p=0.006$) Table (3).

Overall survival: The median follow-up duration for all patients was 18 months ranging from one to 28 months. No significant relationship was found between the initial variables of age, sex, histopathologic subtypes, IPT and overall survival ($p=0.276$, 0.432, 0.139 and 0.698, respectively), however there was a significant relationship with grades of lymphoma ($p=0.015$). In addition, no significant relationship was observed between ICAM-1 or CD44s expression and overall survival. ($p=0.869$ and

0.803, respectively). When an optimum cut-off value was used, the overall survival for patients with sICAM-1 levels < 600 ng/ml was 86% and for patients with serum levels of sICAM-1 ≥ 600 ng/ml was 81% and the difference was not significant ($p=0.937$). Table (4), Figs. (1-3).

Table (1): Clinical and histopathologic characteristics of the 34 D-NHL patients studied.

Parameter	N (%)
Age, (mean \pm SD) years	46 \pm 11.1
Less than 60	29 (85.2)
More or equal 60	5 (14.5)
Sex:	
Male	22 (64.7)
Female	12 (35.2)
Histologic subtypes:	
Lymphoblastic lymphoma	13 (38.2)
Burkitt's lymphoma	5 (14.7)
Diffuse large cell lymphoma	1 (2.9)
Small lymphocytic lymphoma	14 (41.1)
Mantel cell lymphoma	1 (2.9)
IPT:	
B-cell	30 (88.2)
T-Cell	4 (11.7)
Grade:	
High-grade	19 (55.8)
Low-grade	15 (44.1)
ICAM-1 expression	6 (17.6)
CD44s expression	13 (38.2)
sICAM-1 (median, range) ng/ml	1160 150-2500

Table (2): ICAM-1 and CD44s expression in low and high grade lymphomas.

Variable	Low-grade (n=15) No and %	High-grade (n=19) No and %	<i>p</i> -value
ICAM-1	2 (13.3)	4 (21)	0.672
CD44s	4 (26.6)	9 (47.3)	0.296

Table (3): The levels of sICAM-1 in D-NHL cases and healthy controls.

Lymphoma	sICAM-1 (ng/ml) Median-Range	<i>p</i> -value
Low grade N=19	1440 380-2500	
High grade N=15	890 150-1540	0.006
Control N=10	375 270-620	

Group means sharing same letter are not statistically significant in pair wise comparison.

Table (4): The relation of overall survival with different studied variables.

Variable	Overall survival		p-value
	N	%	
Age:			
Less than 60	29	79	0.276
More or equal 60	5	100	
Sex:			
Male	22	86	0.432
Female	12	74	
Histopathologic subtypes:			
Lymphoblastic	13	69	0.139
SLL	14	100	
Others*	9	78	
IPT:			
B-cell	30	83	0.698
T-cell	4	75	
Grade:			
High	19	67	0.015
Low	15	100	
ICAM-1:			
Positive	6	83	0.869
Negative	28	82	
CD44s:			
Positive	13	38	0.803
Negative	21	81	
sICAM-1:			
≥600 ng/ml	27	81	0.937
<600 ng/ml	7	86	

* Others = 1 BL, 2 DLCL, and 3 MCL.

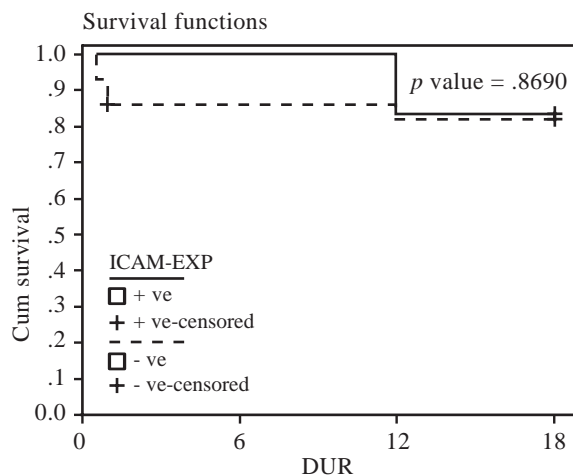


Fig. (1): Overall survival and ICAM-1 expression.

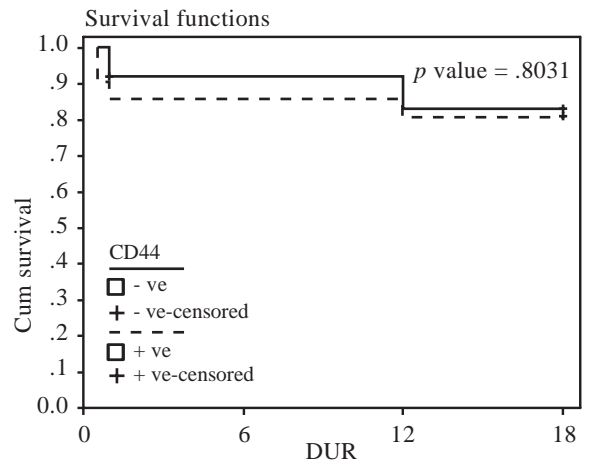


Fig. (2): Overall survival and CD44s expression.

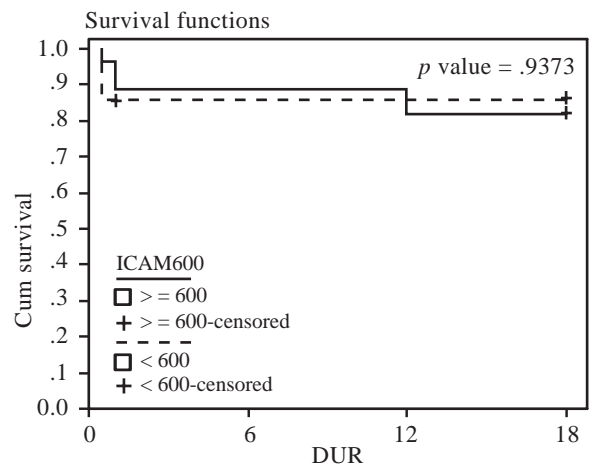


Fig. (3): Overall survival and sICAM-1 level.

DISCUSSION

Regulated lymphocyte trafficking is essential for the control and integration of the immune response. The homing process disperses the immunologic repertoire, guides lymphocyte subsets to the specialized environment that control their differentiation and survival, and targets immune effector cells to sites of antigenic insults [3].

The adhesion receptors regulating trafficking of normal lymphocytes are also expressed and functionally active in their malignant counterparts, the non-Hodgkin's lymphomas. These homing receptors appear to mediate highly tissue-specific dissemination of specific lymphoma subtypes, such as lymphomas of mucosa associated lymphoid tissue and lymphomas of the skin. Furthermore, as a result of their capability to enhance lymphoma dissemination and

transducer signals into the cell, promoting cell growth and survival, adhesion receptors may contribute to lymphoma aggressiveness [8].

The aim of the present study was to study the expression of two adhesion molecules (CD44s and ICAM-1) and the serum level of sICAM-1 in a group of patients with D-NHL and to correlate the findings with patients overall survival.

In the present study, most of our NHL patient's histopathologic types were lymphoblastic lymphoma (38.2%) and SLL (41.1%). This high incidence can be explained by the high rate of dissemination to the bone marrow of these types of NHL at time of diagnosis.

In the present work ICAM-1 was expressed in 6/34 cases (17.6%) of D-NHL. This low level of expression was explained by Terol et al., [9] who studied 70 cases of NHL and found that patients with weak or negative expression had more frequently disseminated (stage-4) disease, extranodal involvement and bone marrow infiltration than those with localized disease.

As regard histopathologic types of lymphoma cases, ICAM-1 was positive in 1/14 of SLL, and in MCL (n=1). Our results were similar to those of Jacob et al., [10] who studied 24 cases of SLL, 15 MCL and 39 follicular lymphoma and measured cell adhesion molecule expression by the mean value of green fluorescence rather than reporting the percentage of positive cells. The lowest expression of ICAM-1 was among low and intermediate grade lymphoma. They concluded that, low grade NHL which are derived from quiescent cells and show indolent course of disease express low levels of ICAM-1. Our result were also consistent with those of Csanaky et al., [11] who studied 39 cases of SLL and found that the proportion of ICAM-1 positive cells is significantly lower ($p < 0.001$) in SLL compared to non-SLL. A weak ICAM-1 expression, as found in circulating normal lymphocytes, could explain in part, the tendency of indolent lymphoma to leukemic dissemination through a deficient homotypic adhesion [8].

In high grade lymphoma, ICAM-1 was expressed in (21%) of cases, all of them were lymphoblastic lymphoma. ICAM-1 was negative in all cases of BL and DLCL. In the literature there was a controversy regarding ICAM-1

expression in high grade lymphoma. Our results were similar to those of Vecca et al., [12] who found partial or total loss of ICAM-1 epitopes in aggressive lymphomas especially BL and LBL. They reported that down regulation of ICAM-1 could be involved in the rapid progression of these types of lymphoma. Similar results were reported by Jacob et al., [11] who related the rapid progression of BL to their defect in adhesion molecule expression which may explain their escape from the immune system. On the other hand Maio et al., [13] reported that high grade B-cell lymphomas express in general, a high level of ICAM-1 than low grade lymphomas. Moreover, Yaris et al., [1] studied diffuse high grade lymphoma and reported ICAM-1 expression on blast cell surface in eight out of twelve cases.

Expression of ICAM-1 on leukemic cells renders them susceptible to NK- or LAK-cell mediated lyses, and therefore diminishes their number. In vitro data have suggested a role for ICAM-1 in mediating cytolytic activity toward tumor cells. Leukemic blasts might acquire a selective advantage by down-regulating or shedding ICAM-1 and therefore escape from host immunosurveillance and subsequently lymphoma dissemination. In contrast, localized large cell lymphoma which frequently present with localized disease tend to express ICAM-1 [2].

In this work we had four cases of mediastinal lymphoma and none of them expressed ICAM-1. Our results were consistent with that of several investigators [1,13,14] who agreed that ICAM-1 is not detected on malignant cells of patients with T-cell LBL.

No significant relation was found between ICAM-1 expression and overall survival in our study. These results were similar to those of Yaris et al., [1] who studied 20 patients with NHL and found no significant relation between ICAM-1 expression and the overall survival. On the other hand, Terol et al. [9] studied ICAM-1 expression in 70 patients with non-Hodgkin's lymphoma and found that patients with positive ICAM-1 expression had significantly better survival rates than those with negative or weak expression of ICAM-1 (2 year overall survival: 77% vs, 50% respectively; $p < 0.025$). The non significant results in this work and that of Yaris et al., [1] could be related to the small number of patients.

In literature, there is a controversy on CD44s expression of NHL. We observed 26.6% expression in low-grade D-NHL, these included 3/14 SLL, and 1/1MCL. Our results were close to those of Aguilera et al., [15] who studied 30 cases of SLL and 20 cases of MCL using immunohistochemistry on paraffin sections and found that CD44s was strong positive in 3/30 cases of SLL (10%), and 15/20 of MCL (75%). On the other hand, Jewell and Yong, [16] using flow cytometric analysis of CD44s reported that all samples analyzed expressed high levels of CD44s. The controversy between results reported by different authors could be related to different methodology.

In high-grade D-NHL, we found that CD44s was expressed in (47%) cases. The results of the present work were comparable to those of Horst et al., [17] who found that CD44s was expressed in 51% of patients with tumor spread beyond stage II. Also Yaris et al., [1] on using monoclonal antibody directed against CD44 constant region found that half of high grade NHL had negative CD44 expression.

The correlation of CD44s expression with overall survival in the literature showed contradiction. Yaris et al., [1] found no significant relation between CD44s expression and overall survival. In the present work, we found similar results. On the other hand, Drillenburger and pals [3], studied a single group of lymphoma (DLCL) and found that CD44s expression was an important prognostic factor. However, in addition to CD44s, NHLs may express CD44 isoforms containing variant exons. These larger splice variants appear to be predominantly expressed on a subgroup of aggressive lymphomas. They often contain exon v 6/7 encoded sequences which have been reported to confer metastatic behavior in rat carcinoma cell line [18].

The serum levels of sICAM-1 were significantly elevated in all patients with D-NHL compared to healthy control ($p < 0.001$) in the present work. Perez- Encinas et al., [2] measured sICAM-1 in 63 patients with non-Hodgkin's lymphoma (NHL) and found that the serum levels of sICAM-1 were significantly elevated in patients with NHL compared to healthy controls. The latter study support our results.

Patients with D- low grade NHL had a higher levels of sICAM-1 compared to that of patients

with D-high grade NHL in the present work and the difference was statistically significant ($p = 0.006$). The higher level of sICAM-1 in these patients may be explained by the presence of 14 cases of D-SLL (CLL) out of 15 low grade NHLs. And all these 14 cases were in the stage III and IV using the Rai-clinical staging of B-CLL. Christiansen et al., [19] reported that the serum levels of sICAM-1 in B-CLL were positively correlated with tumor mass as reflected by the Rai and Binet staging systems. The latter findings may explain our observation.

The serum level of sICAM-1 was correlated with overall survival and prognosis by some authors, [20,21] who found that NHL patients with sICAM-1 over 600 ng/ml had a significant shorter overall survival. Here we found that patients with sICAM-1 level ≥ 600 ng/ml had shorter overall survival than those with levels < 600 ng/ml although the difference was statistically not significant, ($p = 0.9$). Small sample size ($n = 34$) may explain the non-significant results in this work.

The cellular origin of ICAM-1 in NHL is presently unknown, although it has been suggested that sICAM-1 is released from the cell surface by proteolytic cleavage [22]. The cell surface expression of ICAM-1 is reported to vary according to the maturation stage of the B-cells [13,14,23]. Data on low grade NHL suggest that the lack of cell surface expression of ICAM-1 is associated with a leukemic course of the disease [24]; whereas this is not the case in high grade lymphoma [17]. Serum ICAM-1 has been shown to be released by cytokine stimulated human melanoma cells. Thus a possible source for sICAM-1 in NHL could be the malignant cells themselves, but since all NHL subgroups have elevated levels of sICAM-1 and the expression of surface ICAM-1 may be variable, it seems unlikely that a simple proteolytic cleavage is the origin of sICAM-1 in all subgroups. Other possible sources for sICAM-1 could be regulatory cells such as T and NK cells or activated endothelial cells [20].

Thus we conclude that, D-NHL express low levels of ICAM-1. In low-grade NHLs, which are derived from quiescent cells and show indolent course of disease, there was low levels of ICAM-1 expression. In high grade lymphoma, which are derived from proliferating cells and clinically aggressive, down regulation of ICAM-

1 explained their escape from immunosurveillance and subsequently their rapid progression. ICAM-1 was not detected on malignant cells of patients with T-cell lymphoblastic lymphoma. There is still a controversy regarding CD44s expression in low-grade NHL but in high-grade NHL there was a correlation between CD44s expression and lymphoma dissemination. No correlation was found between ICAM-1, CD44s expression and overall survival. All NHL subgroups have elevated levels of sICAM-1. Serum levels of sICAM-1 in D-SLL correlates with the tumor mass as reflected by the Rai and Binet staging systems. Patients with sICAM-1 level ≥ 600 ng/ml had shorter overall survival than those with levels < 600 ng/ml although the difference was statistically not significant.

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