Prognostic Utility of Transforming Growth Factor Beta-1 in Diffuse Large Cell Non-Hodgkin Lymphoma

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Abstract

Background: Non-Hodgkin lymphomas (NHLs) are heterogeneous group of lymphoproliferative malignancies with different patterns of behavior and response to treatment that usually originate in lymphoid tissues and can spread to other organs. The aim of the work was to evaluate transforming growth factor beta-1 (TGF-β1) in diffuse large cell B-cell lymphoma and response to R-CHOP protocol of therapy.

Methods: This study had been conducted on 50 patients with diffuse large B-cell lymphoma, their ages ranged from 18 to 60 years with a mean age of 44.5 ± 10.7 years. Ten age- and sex-matched apparently healthy individuals were included as control group and the diagnosis and staging of diffuse large B-cell lymphoma was based on clinical, radiological and histopathological criteria.

Results: Our study revealed that soluble TGF-β1 was significantly elevated in comparison to control group (P < 0.001), and it was correlated with advanced stages, bulky disease, high risk international prognostic index, and partially or non-responded patients (r = 0.6, 0.8, 0.3, and 0.2, respectively), TGF-β1 which was high in all patients. It was an independent risk factor for disease-free survival (DFS) (P = 0.007, hazard ratio (HR): 3.5) and overall survival (OS) (P = 0.003, HR: 5.8), along with poor performance status (PS); patients with high TGF-β1 initially showed inferior survival curves in non-responded compared with responded patients for treatment in OS (2-year OS of 72%, P < 0.001) and DFS (2-year DFS of 54%, P < 0.00).

Conclusion: A significant relation was detected between TGF-β1 and treatment response as well as survival indicating its promising value as a prognostic and predictive marker for treatment outcome and survival.

Keywords: Diffuse large B-cell lymphoma; Transforming growth factor beta-1; Prognosis

Introduction

Non-Hodgkin lymphomas (NHLs) were a heterogeneous group of lymphoproliferative malignancies with different patterns of behavior and response to treatment that usually originate from lymphoid tissues and could infiltrate other organs [1]. In Egypt, NHL was the fifth most common cancer in both sexes, the general incidence rate of NHL was 5.90 in 1995 and reached 8.99 in 2004, with a peak (9.40) in the year 2002, with male predominance. As regards the population age from 15 to 60 years old, there was a rise through the period from 1994 to 1999, then a drop from 2000 to 2004. In the elderly group, the incidence rate was doubled during the 10 years [2, 3]. Diffuse large B-cell lymphoma was a fast-growing, aggressive form of NHL and although there were more than 30 types of NHL, diffuse large B-cell lymphoma was the most common type, and constituted about 30-40% of all lymphomas [4]. Transforming growth factor beta-1 (TGF-β1) was a multifunctional protein that regulates proliferation, migration, survival, differentiation, and extracellular matrix synthesis in endothelial cells and vascular smooth muscle cells, as well as in the maintenance of vascular homeostasis [5].

TGF-β1 played a curial role in tumor biology, and the TGF signaling pathway had a controversial role in regulating normal hematopoiesis. During the pathogenesis of hematologic malignancies, normal homeostatic mechanisms regulating cellular proliferation, differentiation, and apoptosis became disrupted due to mutation of transforming growth factor receptor I, II leading to angiogenesis alteration of stromal environment with local and systemic immunosuppression [6].

This work was aimed to evaluate soluble TGF-β1 in diffuse large cell NHL patients and response to R-CHOP protocol of therapy.

Patients and Methods

This study had been carried out in the Hematology/Oncology Unit, Internal Medicine Department, Clinical Pathology Depart-
Methods

All patients were subjected to the clinical evaluation (detailed history with stress on presence or absence of B symptoms, full clinical examination including surface area, general examination, measurement of palpable lesions, lymph node examination with stress on sites and numbers and evaluation of PS according to ECOG), laboratory evaluation (complete blood count (CBC), liver function tests, renal function tests, lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), serum uric acid, serum electrolytes and bone marrow examination by bilateral iliac bone biopsy), radiological examination (chest X-ray and computed tomography (CT) if there is any abnormality in the plain film, CT abdomen and pelvis, radio nucleotide bone scanning for suspected or established bony involvement, electrocardiography (ECG), and echo cardiography (ECHO)) and special investigation for TGF-β1. All patients included in study were treated by R-CHOP protocol (50 mg/m² doxorubicin (adriamycin) on day 1, 375 mg/m² rituximab per cycle) after approval of local ethical committee and all patients were reassessed for response of treatment according to response criteria [7].

Method of assay

Quantification of TGF-β1 was done using ELISA (RayBio-Tec).

1) All reagents and samples brought to room temperature (18 - 25 °C) before use. It was recommended that all standards and samples be run at least in duplicate.

2) 100 µL of each standard and sample added into appropriate wells and covered well with incubation for 2.5 h at room temperature or overnight at 4 °C with gentle shaking.

3) Discard the solution and wash four times with 1 × wash solution. Wash by filling each well with wash buffer (300 µL) using a multi-channel pipette or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4) 100 µL of 1 × prepared biotinylated antibody added to each well and incubated for 1 h at room temperature with gentle shaking.

5) 100 µL of prepared streptavidin solution added to each well and incubated for 45 min at room temperature with gentle shaking.

6) 100 µL of TMB one-step substrate reagent (item H) added to each well and incubated for 30 min at room temperature in the dark with gentle shaking.

7) 50 µL of stop solution (item I) added to each well. Read at 450 nm immediately.

Data analysis

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Sensitivity

The minimum detectable level of TGF-β1 was typically less than 8 pg/mL with intra-assay and CV more than 10% and inter-assay less than 12% [8].

Statistical analysis

Data were analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). Numerical data of scores were expressed as mean and standard deviation or median and range as appropriate. Chi-square test (Fisher’s exact test) was used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Spearman-rho method was used to test correlation between numerical variables. Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. Cox-regression method was used to test the relation between numerical variables and survival. Odds ratio (OR) with its 95% confidence interval (CI) was used for risk estimation. The receiver operating characteristic (ROC) curve was used for prediction of cutoff values and P-value < 0.05 was considered significant.

Results

Fifty patients with diffuse large cell NHL (32 males and 15 females) were included in the study and their age ranged from 18 to 60 years with mean age of 44.5 ± 10.7 years. Twenty-three patients were stage I or II by clinical and radiological assessment, most of the patients (82%) were low or intermediate risk according to international prognostic index, only 11 patients...
had PS ≥ 2, lymphadenopathy, splenomegaly, anemia were the most common clinical presentations observed in all patients and hepatitis C virus infection was associated with 30% either alone or concomitant with hepatitis B infection as shown in Table 1. All of them were treated with R-CHOP protocol of therapy and followed for 2 years after end of treatment and 84% of patients achieved complete remission (CR).

TGF-β1 was correlated with advanced stages, bulky disease, high risk international prognostic index, and partially or non-responded patients (r = 0.6, 0.8, 0.3, and 0.2, respectively) (Table 2).

Multivariate analysis revealed that the TGF-β1, which was high in all patients, was an independent risk factor for disease-free survival (DFS) (P = 0.007, hazard ratio (HR): 3.5) and overall survival (OS) (P = 0.003, HR: 5.8) as shown in Table 3 and the patients with high TGF-β initially showed inferior survival curves in non-responded compared with responded patients for treatment in OS (2-year OS of 72%, P < 0.001; Fig. 1) and DFS (2-year DFS of 54%, P < 0.001; Fig. 2).

Discussion

Diffuse large B-cell non-Hodgkin lymphoma (DLBCL) was the commonest histological subtype of NHL accounting for approximately 30-40% of new diagnoses in adult patients and it had an aggressive behavior. CRs could be achieved in about half of the patients. However, long lasting remissions were not achieved in two-thirds of patients [9].

TGF-β1 was a pleiotropic cytokine that played a pivotal role in regulating cell growth and differentiation in a variety of cell types and it can be expressed in a secreted form or be present on the cell surface in a membrane-bound form [10].

The role of TGF-β1 in immune response had attracted much attention due to the finding that TGF-β was important in proliferation and maturation of T regulatory cell [11].

In the malignant scenario, innate and adaptive immune cells functions were suppressed by tumor-derived TGF-β1, so tumor escape from host immune surveillance [12]. Also the role of TGF-β1 in malignant invasion of many cancers was documented in various types of malignancies including breast cancer [13], prostatic cancer [14], pleural cancer [15] and skin cancer [16].

The majority of the studied patients were males 64.0% and this was in accordance with the study of Goldman et al.
who found that 61.9% of Egyptian patients with diffuse large B-cell lymphoma were males. Lymphadenopathy was the commonest reported clinical presentation (26.0%) followed by splenomegaly (16.0%) and anemia (14.0%) and these well-documented presentations for DLBCL [18]. Hepatitis C virus had not only hepatotropic but also lymphotropic effect and hepatitis C infection was reported in 24.0% of patients while hepatitis B infection was reported in 2% and co-presentation of HBV and HCV was reported in 6.0% of patients. The rate of HCV co-occurrence with DLBCL was relatively low when compared with the study of Goldman et al [17] and this might attribute to smaller sample size in our study and other study (n = 486). Hepatitis B virus infection was also reported with DLBCL according to Wang et al [19].

A soluble TGF-β1, secreted by both lymphoma cells and intratumoral T cells, was observed in the serum of patients with B-cell NHL and TGF-β1 promoted regulatory T (T reg) cells by enhancing expression of Foxp3 in CD4+ T cells and suppressed effector helper T (TH) cells by inhibiting expression of IFN-γ and IL-17 and TGF-β1 was able to bind to the surface of lymphoma B cells through its interaction with heparin sulfate (HS) but not through the TGF-β receptor leading to proliferation and cytokines production in malignant B-cell lymphoma [13].

TGF-β1 was elevated before treatment in patient with DLBCL and dramatically decreased after treatment and correlated with advanced stages, high risk international prognostic index, bulky disease and partially or non-responded patients so it is linked to all bad prognostic factors, complete response was achieved in 42 patients (84.0%) while four patients (8.0%) had partial remission and four patients (8.0%) had no response to protocol of chemotherapy and these data were consistent with the study of Bedewy et al [20], in which 178 Egyptian patients (79.5%) achieved complete response after the R-CHOP regimen with follow-up period of 51 months.

The addition of rituximab to the cyclophosphamide, dox-

### Table 3. Multivariate Analysis for Survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>Disease free survival</th>
<th>Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P value</td>
<td>HR</td>
</tr>
<tr>
<td>Age</td>
<td>0.57</td>
<td>0.8</td>
</tr>
<tr>
<td>Performance status ≥ 2</td>
<td>0.03</td>
<td>3.4</td>
</tr>
<tr>
<td>Elevated LDH</td>
<td>0.86</td>
<td>0.1</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>0.007</td>
<td>3.5</td>
</tr>
<tr>
<td>B symptoms</td>
<td>0.53</td>
<td>0.7</td>
</tr>
<tr>
<td>Clinical stage</td>
<td>0.43</td>
<td>1.4</td>
</tr>
<tr>
<td>Bulky disease</td>
<td>0.008</td>
<td>3.7</td>
</tr>
</tbody>
</table>

CI: confidence interval; PFS: progression-free survival; HR: hazard ratio; OS: overall survival.

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Figure 1. Two-year overall survival curve of patients with diffuse large cell non-Hodgkin lymphoma.
orubicin, vincristine, and prednisone (CHOP) regimen has greatly improved outcomes for patients with DLBCL and this regimen confers two major benefits, a decrease in the number of patients with disease progression during treatment (refractory patients), and also decrease in the number of relapsing patients [21].

A significant higher level of TGF-β1 was observed before treatment with significant decline after treatment specially in complete responded patients [22] reflecting it is a predictive value of it and this was supported by Tas et al, who determine a significance reduction of the serum level of TGF-β1 in epithelial ovarian cancer patients after chemotherapy with persistent elevation in non-responded one [23], and a significant decline after treatment specially in complete responded patients might attributed to immunomodulatory effect of this combined regimen of chemotherapy especially cyclophosphamide which caused a significant decrement of Foxp3 expression, decreased percentage of CD4CD25 double positive cells and impaired spontaneous molecular release of TGF-β with enhancement of anti-tumor immunity and improved therapeutic outcome [24, 25].

**Conclusion**

TGF-β1 in patients with DLBCL had a significant association with other bad prognostic factor and disease outcome and a significant decline of its level after treatment especially in responded patients was an indicative of its promising value as a prognostic and predictive value for treatment outcome and survival. Also targeting of TGF-β1, its receptors or Foxp3 expression cells might open a future direction in treatment of DLBCL.

**Conflicts of Interest**

There was not any conflicts of interest for author.

**References**


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