

B-lymphocyte stimulator: can we consider it a marker for severity of hepatitis C virus-induced B-cell non-Hodgkin lymphoma?

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Objective

B-lymphocyte stimulator (BLyS) is a member of tumor necrosis factor family. BLyS is essential for the survival of normal and malignant B lymphocyte. Hepatitis C virus (HCV) infection likely represents the early event leading to BLyS upregulation. The aim of this study was to determine the relation between serum BLyS levels and the severity of HCV-related B-cell non-Hodgkin lymphoma (B-NHL).

Patients and methods

Seventy-eight B-NHL patients both HCV-positive and HCV-negative and 20 patients with HCV infection without lymphoproliferative disorders, in addition to 20 age-matched and sex-matched controls, were included in the study. PCR for HCV, evaluation of levels of soluble BLyS protein in blood, bone marrow aspirate and biopsy with flow cytometry, and lymph node biopsy with immunophenotyping for CD5, CD23, CD10, CD20, and cyclinD1 were performed.

Results

The serum BLyS levels were significantly higher in B-NHL patients and HCV patients without lymphoproliferative disorders compared with the control group. The serum BLyS levels were statistically significantly higher in aggressive lymphoma patients with HCV-positive infection compared with aggressive lymphoma patients with HCV-negative infection, but there was no statistically significant difference between BLyS levels in indolent B-NHL patients with or without HCV infections. Moreover, there was no statistically significant difference between BLyS levels in aggressive and indolent lymphoma patients with HCV-positive infection.

Conclusion

BLyS levels are increased in HCV-induced B-NHL but it cannot be considered as a marker for severity of the disease (indolent or aggressive). More studies are needed.

Keywords:

B cell, B-cell-activating factor, B-cell non-Hodgkin lymphoma, B-lymphocyte stimulator, hepatitis C virus, lymphoma

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Introduction

Egypt has the highest hepatitis C virus (HCV) prevalence in the world with an estimated prevalence of 14.7% among those between 15 and 59 years of age [1–4].

HCV carriers are prone to development of chronic hepatitis, cirrhosis, and hepatocellular carcinoma [5]. Moreover, long-standing infection with HCV can lead to the development of extrahepatic manifestations of the disease, such as mixed cryoglobulinemia and other immune complex-mediated disorders due to B-cell proliferation that may finally evolve into overt B-cell non-Hodgkin's lymphoma (B-NHL) [6,7].

Farawela *et al.* [8] noticed a significantly higher prevalence of HCV infection among newly diagnosed

NHL patients before therapy relative to controls, and this conferred a 14-fold increased NHL risk with HCV infection.

It has been suggested that HCV plays an important role in the pathogenesis of B-NHL as many non-Hodgkin's lymphoma (NHL) subtypes are associated with HCV infection, such as diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), and lymphoplasmacytic lymphoma [9].

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It has been postulated that HCV is responsible for B-lymphocyte stimulator (BLyS) upregulation, which acts as a stimulus for B-cell autoimmunity and lymphoproliferation in infected patients [10].

BLyS, also called B-cell-activating factor, is a member of tumor necrosis factor family, which causes B-cell growth stimulation [11]. It is expressed in a variety of cells types, such as peripheral blood leukocytes and stromal cells of the spleen and lymph nodes [12,13]. It is an important factor affecting B-cell proliferation, survival, maturation, and antibody production [11].

Abnormal production of BLyS in NHL by malignant cells themselves or by surrounding microenvironment can protect these cells from programmed spontaneous death and promote their growth and survival [14].

Thus, the aim of this study was to estimate BLyS levels in patients with HCV-induced B-NHL and correlate it with the severity of the disease as aggressive or indolent lymphoma.

Patients and methods

This prospective nonrandomized study was conducted at the Clinical Hematology Unit, Internal Medicine Department, Faculty of Medicine, and South Egypt Cancer Institute, Assiut University, between January 2014 and December 2016. This study included 78 patients with B-NHL (group I), both HCV-positive ($n=25$, 32.1%) and HCV-negative ($n=53$, 67.9%). Their ages ranged from 17 to 75 years with a mean of 50.56 ± 1.62 years. According to REAL classification 2008, from the total 78 patients of group I, 33 (42.3%) patients were diagnosed with indolent lymphoma (CLL/SLL), whereas the remaining 45 (57.7%) patients were diagnosed with aggressive lymphoma (42 patients with DLBCL and three patients with MCL).

The study included 20 patients (group II) with HCV infection without lymphoproliferative disorders. Their ages ranged from 21 to 68 years with a mean of 43.17 ± 1.35 years and a median of 45 years. In addition, there were 20 age-matched and sex-matched healthy individuals as a control group, including 16 male and four female participants; their ages ranged from 20 to 69 years with a mean of 43.17 ± 1.35 years and a median of 44 years. Written informed consent was taken from all participants; this study was approved by the ethical committee of Assiut University. All patients and controls were evaluated by means of history taking, clinical examination, complete blood count and erythrocyte sedimentation rate, chest radiography, liver

and kidney function tests, and abdominal ultrasound. HCV infection was screened by searching for serum antibodies against HCV using enzyme linked immune sorbent assay (ELISA) and PCR and the levels of soluble BLyS protein in peripheral blood was measured using antigen-capture ELISA. To all patients with NHL (78 patients), the following investigations were performed: bone marrow aspirate and biopsy with flow cytometry, computed tomography scan of the chest, abdomen, and pelvis, and lymph node biopsy with immunophenotyping for CD5, CD23, CD10, CD20, and cyclinD1.

Statistical analysis

Data entry and data analysis were performed using statistical package for social science, SPSS for Windows, Version 16.0. Chicago, SPSS Inc. Data were presented as number, percentage, mean, SD, SE, median, and range. The Mann-Whitney test was used to compare quantitative variables between two groups. *P*-value was considered statistically significant when *P*-value is less than 0.05.

Results

This study included 78 patients with B-NHL (group I), both HCV-positive and HCV-negative, and 20 patients (group II) with HCV infection without lymphoproliferative disorders, in addition to 20 age-matched and sex-matched healthy individuals as a control group.

From the total 78 patients (group I) with B-NHL, 33 (42.3%) patients were diagnosed with CLL/SLL, 42 (53.8%) patients with DLBCL, and the remaining three (3.8%) patients with MCL. From the 33 patients with CLL/SLL, nine patients were positive for HCV infection, whereas the remaining 24 patients were negative, and of 42 patients with DLBCL 14 patients had positive HCV infection, whereas 28 patients were negative. Two patients of those diagnosed with MCL had positive HCV infection, whereas one patient was negative for HCV infection.

The serum BLyS levels were significantly increased in group I (median: 6.8 ng/ml, mean \pm SE: 11.68 ± 1.78 , range: 1.1–64 ng/ml) and group II (median: 7 ng/ml, mean \pm SE: 6.49 ± 0.41 , range: 3.4–9 ng/ml) when compared with the control group (median: 3.2 ng/ml, mean \pm SE: 3.13 ± 0.25 , range: 0.8–5 ng/ml) with *P*-value less than 0.01 for both comparisons (Tables 1 and 2).

There were no statistically significant differences in serum BLyS levels in aggressive lymphoma patients (DLBCL and MCL) as the median of BLyS levels was

7.5 ng/ml (mean±SE: 11.24±2.63, range: 1.1–60 ng/ml) when compared with the serum BLYS levels in indolent lymphoma patients (CLL/SLL) as the median of the serum BLYS levels was 5.8 ng/ml (mean±SE: 11.15±2.44, range: 1.2–64 ng/ml) with *P*-value equal to 0.567 (Table 3).

The serum BLYS levels were statistically significantly higher in aggressive lymphoma patients with HCV-positive infection (median: 7.8 ng/ml, mean±SE: 15.21±5.56, range: 3.6–64 ng/ml) when compared with aggressive lymphoma patients with HCV-negative infection (median: 5.5 ng/ml, mean±SE: 9.66±2.77, range: 1.2–62) with *P*-value equal to

Table 1 The relation between serum B-lymphocyte stimulator levels in non-Hodgkin lymphoma patients and control group

	Mean±SE	Median	Range	<i>P</i> -value
NHL patients	11.68±1.78	6.8	1.1–64.0	<0.01
Control	3.13±0.25	3.2	0.8–5.0	

NHL, non-Hodgkin lymphoma.

Table 2 Relation between serum B-lymphocyte stimulator levels in hepatitis C virus infected patients and control group

BLYS	HCV (<i>n</i> =20)	Control (<i>n</i> =20)	<i>P</i> -value
Mean±SE	6.49±0.41	3.13±0.25	<0.01
Median (range)	7.0 (3.4–9.0)	3.2 (0.8–5.0)	

BLYS, B-lymphocyte stimulator; HCV, hepatitis C virus.

Table 3 Serum B-lymphocyte stimulator levels in indolent and aggressive lymphoma patients

BLYS	Mean±SE	Median	Range	<i>P</i> -value
Aggressive lymphoma patients (DLBCL and MCL)	11.24±2.63	7.5	1.1–60.0	0.567
Indolent lymphoma patients (CLL/SLL)	11.15±2.44	5.8	1.2–64.0	

BLYS, B-lymphocyte stimulator; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma.

Table 4 The relation between serum B-lymphocyte stimulator levels in indolent lymphoma patients and aggressive lymphoma patients (positive and negative hepatitis C virus infection)

B-cell NHL patients	BLYS levels (ng/ml)		<i>P</i> -value
	Median (range)	Mean±SE	
Indolent lymphoma patients (<i>n</i> =9 CLL/SLL) with HCV-positive infection	6.8 (2.3–52)	11.09±5.19	0.671
Indolent lymphoma patients (<i>n</i> =24 CLL/SLL) with HCV-negative infection	7.7 (1.1–60)	12.81±3.36	
Aggressive lymphoma patients (<i>n</i> =14 DLBCL+2 MCL) with HCV-positive infection	7.8 (3.6–64)	15.21±5.56	0.041
Aggressive lymphoma patients (<i>n</i> =28 DLBCL+1 MCL) with HCV-negative infection	5.5 (1.2–62)	9.66±2.77	

BLYS, B-lymphocyte stimulator; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; HCV, hepatitis C virus; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma.

Table 5 The relation between serum B-lymphocyte stimulator levels in indolent and aggressive lymphoma patients with positive hepatitis C virus infection

B-cell NHL patients	BLYS levels (ng/ml)		<i>P</i> -value
	Median (range)	Mean±SE	
Indolent lymphoma patients (<i>n</i> =9 CLL/SLL) with HCV-positive infection	6.8 (2.3–52)	11.09±5.19	0.541
Aggressive lymphoma patients (<i>n</i> =14 DLBCL+2 MCL) with HCV-positive infection	7.8 (3.6–64)	15.21±5.56	

BLYS, B-lymphocyte stimulator; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; HCV, hepatitis C virus; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma.

0.041. However, there were no statistically significant differences in indolent lymphoma patients with HCV-positive infection (median: 6.8 ng/ml, mean±SE: 11.09±5.19, range: 2.3–52 ng/ml) when compared with indolent lymphoma patients with HCV-negative infection (median: 7.7 ng/ml, mean±SE: 12.81±3.36, range: 1.1–60 ng/ml) with *P*-value equal to 0.671 (Table 4).

However, there was no statistically significant difference in BLYS levels between indolent and aggressive lymphoma patients with positive HCV (Table 5).

Discussion

HCV infection is the most common chronic viral infection worldwide affecting about 180 million people, which accounts for 3% of the world population and it is endemic in Egypt [15].

BLYS, a tumor necrosis factor family member, is a very important factor in B-cell survival and maturation [16]. It has been suggested that HCV infection may present an early stimulator to BLYS upregulation, leading to progression into B-NHL [10].

In the current study serum BLYS levels were significantly higher in patients with HCV infection

without lymphoproliferative disorders compared with controls ($P<0.01$). This is in agreement with previous reports such as that by Fabris *et al.* [10], who found that 10/33 (30.3%) of patients with HCV infection have high serum BLYS levels ($P=0.0026$) compared with controls, and Ito *et al.* [17], who reported elevated serum BLYS levels in patients with HCV than in controls ($P<0.001$) and they added that HCV infection may induce the release of BLYS.

In our results, serum BLYS levels were significantly elevated in patients with B-NHL when compared with the control group ($P<0.01$). Our results are in agreement with Yang *et al.* [18], who found that serum BLYS levels were significantly elevated in patients with most NHL compared with those in healthy donors, and it correlated with aggressive disease and a poor response to therapy. Haiat *et al.* [19] also detected that the levels of soluble BLYS in patients with different NHL subtypes were significantly elevated when compared with those in healthy controls.

In our results we detected increased BLYS levels in 23 of 33 CLL/SLL, 37 of 42 of DLBCL, and three of three MCL, and thus BLYS levels were detected in all histological subtypes of B-NHL. Our results correlated with that of Novak *et al.* [20], who detected BLYS in five of five MCL, five of five DLBCL, three of four B-cell CLL/SLL and one of five follicular lymphoma; BLYS was detected in all NHL specimens studied with variable levels of BLYS detected within the histological subtypes. However, in their study they found that there were no statistically significant differences between serum BLYS level among the healthy control group, indolent lymphoma patients, and aggressive lymphoma. This is in contrast with our results as we detected significantly elevated levels in patients with B-NHL when compared with the control group ($P<0.01$) and they believed that this was likely due to the reagents used in their ELISA.

In this study serum BLYS levels were higher in aggressive lymphoma patients with HCV-positive infection when compared with aggressive lymphoma patients with HCV-negative infection ($P=0.041$). However, there were no statistically significant differences in indolent lymphoma patients with HCV-positive infection when compared with indolent lymphoma patients with HCV-negative infection ($P=0.671$).

Conclusion

BLYS serum levels are elevated in NHL subtypes suggesting that excessive production of BLYS occurs

in these patients. HCV infection likely represents the early event leading to BLYS upregulation. BLYS can be considered as a marker for HCV-induced B-NHL. However, it does not indicate severity of the disease as there was no significant difference between indolent and aggressive lymphomas with HCV-positive infection.

Recommendations

More studies are needed with a larger number of patients and correlation of BLYS levels with outcome and survival of patients after therapy.

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Conflicts of interest

There are no conflicts of interest.

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