Effect of hepatitis C virus infection on the circulating levels of interleukin (IL)-22, IL-32 and IL-34 in non-Hodgkin lymphoma patients

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Received: 6 June 2022
Accepted: 5 September 2022

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Abstract:

Background: Epidemiological studies have demonstrated an increased risk of non-Hodgkin lymphoma (NHL) in patients suffering from chronic hepatitis C virus (HCV) infection. The increased inflammatory response is a key driver for tumor progression and hence, it may worsen the NHL status, when associated with hepatitis like that occurring in HCV patients.

Aim of the work: This study aimed to assess the serum levels of interleukin (IL)-22, IL-32α and IL-34 as potential inflammatory mediators in the group of NHL lymphoma with or without HCV.

Subjects and methods: A group of 25 NHL patients with HCV along with a group of 31 NHL patients without HCV were enrolled in the study. Serum levels of IL-22, IL-32α and IL-34 were assessed using ELISA assay.

Results: Serum levels of IL-22, IL-32α and IL-34 were significantly ($p<0.001$) elevated in NHL patients with HCV, compared to NHL patients without HCV.

Conclusion: HCV infection may be involved in the development and progression of NHL via increasing the circulating levels of IL-22, IL-32α and IL-34.

Keywords: NHL, HCV, Interleukin-22, Interleukin-32α, Interleukin-34

Introduction:

Non-Hodgkin lymphomas (NHL) are malignancies of the lymphoid tissue, which shift in histologic characteristics, clinical appearances, and etiologic variables [1]. Hepatitis C virus (HCV) is a leading cause of liver-related mortality worldwide. Although HCV is a hepatotropic virus, several studies have shown that HCV may infect organs and tissues other than the liver, including peripheral blood cells, kidney, skin, oral mucosa, salivary glands, pancreatic tissues, heart, gallbladder, intestine, and adrenal gland tissues [2]. Moreover, HCV infection has been implicated in extrahepatic malignancies like NHL, cholangiocarcinoma, pancreatic cancer and oral carcinomas [3].

There is sufficient epidemiologic evidence for concurrent association of HCV with B-cell-NHL. The subtypes of B-cell-NHL that associated with HCV are

marginal zone lymphoma and diffuse large B-cell lymphoma. The foremost persuading prove for a causal relationship between HCV infection and lymphoma is the perception of B-NHL resolution after HCV treatment by antiviral therapy [4]. Three common hypotheses have developed to explain the HCV induced NHL transformation. First, sustained stimulation of lymphocyte receptors by viral antigens. Second, HCV replication in B cells with subsequent oncogenic consequences mediated by intracellular viral proteins. Third, persistent B-cell damage caused by a transiently intracellular virus, which is called the ‘‘hit and run’’ theory [5].

Inflammation is a major driver of tumor development and progression and has been associated with poor prognosis. A quintessential signaling mechanism of inflammation is upregulation and release of the proinflammatory cytokines in the systemic circulation [4]. A possible role of cytokines in the development of NHL is supported by recent reports on NHL risk related to genetic variation in genes encoding proinflammatory and anti-inflammatory cytokines [6]. It was hypothesized that cytokines play important roles in B-cell activation, proliferation, and apoptosis, thus may be related to B-cell-NHL risk [7].

Interleukin (IL)-32 is a recently described proinflammatory cytokine that activates p-38 of mitogen-activated protein kinases and nuclear factor kappa B (NF-κB), resulting in expression of proinflammatory cytokines, such as IL-1β and tumor necrosis factor-alpha (TNF-α) [8]. IL-34 is another proinflammatory cytokine involved in several signaling pathways and participates in a wide array of biological actions [9]. IL-34 is identified as the second colony-stimulating factor (CSF)-1 receptor (CSF-1R) ligand. IL-34 can also bind to receptors of protein-tyrosine phosphatase (PTP)-ζ and syndecan-1. Lastly, IL-22 is the main cytokine of Th22 subset, which belongs to the IL-10 cytokine family [10]. IL-22 binds to a heterodimeric receptor complex that consists of IL-22 receptor (R) 1 and IL-10R2. IL-22 signaling activates STAT3 and induces expression of a variety of antiapoptotic (e.g., Bcl-2, Bcl-xL, Mcl-1) and mitogenic (e.g., c-myc, cyclin D1, Rb2, CDK4) proteins [11, 12].

The role of HCV associated inflammation in the progression of NHL as an extrahepatic malignancy has not been investigated in depth. Accordingly, the aim of this study was to address this issue through assessing the serum levels of ILs-32α, 34 and 22 as possible inflammatory mediators in the groups of NHL patients pre-exposed to HCV infection.

Patients and Methods:

Subjects

This study was carried out on a total of 56 NHL patients who were recruited from Oncology Center of Mansoura University from November 2020 to March 2021. Informed consent was obtained from all participants. Ethical approval was obtained from the Ethical Committee at Faculty of Medicine, Mansoura University (R.21.08.1402). The patients were randomized into two groups as follows:

-Group I comprised of 26 patients with early NHL classified as stage I according to Ann Arbor staging.

-Group II comprised of 26 patients with advanced NHL with HCV infection classified as stage I, II, III or IV according to Ann Arbor staging.

Inclusion criteria:

1- Age of participants not less than 18 years old.

2- Patients were diagnosed with NHL.

Exclusion criteria:

1- Patients with other malignancies and other systemic inflammatory diseases.

2- Pregnant or lactating females.

Clinical investigations:

Both groups were subjected to the following:

1- Recording the medical history of other diseases and the family history of disease.

2- Clinical examination including signs and symptoms of NHL.

3- Radiological assessment: CT scans of the neck, chest, abdomen and pelvis were performed.

4- Histopathological diagnosis: Biopsy was taken from NHL patients from the sites of malignancy including lymph nodes, extra nodal masses or spleen to confirm diagnosis and for proper subtyping and staging of disease. Excisional biopsies were performed in majority of patients (81%), while core needle biopsies were performed in 19% of patients.

Laboratory investigations include:

Complete blood count (CBC)

Kidney function tests: Assessment of serum creatinine level.

Liver function tests: Assessment of serum ALT and AST activities.

Virology screen (HCV Ab, HBsAg, HIV).

Sampling:

- About 10 ml of whole blood was withdrawn from each participant by venipuncture using sterile disposable plastic syringes under complete aseptic technique and divided into three aliquots:

-Two milliliters were collected into blood collection tubes containing K2EDTA for complete blood count (CBC).

-Four milliliters were collected in plain dry tubes; blood was left to clot at room temperature for 30 minutes followed by centrifugation at 4000 rpm for 10 minutes to separate serum. The separated serum was divided into two aliquots; the first portion was used for biochemical investigations of serum ALT and AST activities, and serum creatinine levels, while the second portion was stored at -20 C° for determination of serum levels of IL-22, IL-32α and IL-34.

Enzyme-Linked Immunosorbent Assay (ELISA):

All serum specimens were stored at -20°C used for determination of cytokines. The concentrations of IL-

22, IL-32α and IL-34 in each group were determined with a quantitative sandwich enzyme immunoassay technique in accordance with the manufacturer's recommendations (BioLegend ELISA MAX™ Deluxe, San Diego, CA) using a 96-well plate reader (BioTek ELx800, USA). The concentrations were calculated from a standard curve established under the same conditions for samples.

Results:

I - Demographic data of the studied groups:

The NHL group included 26 patients [13 males (50%) and 13 females (50%)] with a mean age of 49.23 ± 16.15 years. The HCV + NHL group included 26 patients [15 males (57.7%) and 11 females (42.3%)] with a mean age of 55.12 ± 12.77 years (Table 1).

II- Ann Arbor staging of NHL

According to Ann Arbor staging, 26 NHL patients were classified as stage I (they had one involved site of disease). In NHL and HCV group, 1 patient was diagnosed as stage I (had two or more involved sites of disease on the same side of diaphragm) and 5 patients were classified as stage II. Of the remaining patients, 13 were classified as stage III and 7 were classified as stage IV (Table 1).

NHL treatment protocol:

Diffuse large cell NHL patients were aggressively treated with R Chop or R-Epoch lines of chemotherapy and may be consolidated by radiotherapy. Patient refractory for 1st line chemotherapy or relapsed after time of therapy was treated by salvage chemotherapy with DHAP, MINE, ESHAP protocols, followed by autologous bone marrow transplantation (Table 1)

HCV treatment for the patients

In NHL + HCV patients, only 19 patients were assigned to treatment for HCV and the remaining 7 patients received no HCV treatment (Table 1)

III-Routine biochemical analyses:

A significant difference was detected in mean serum AST activity in NHL and HCV group, compared to NHL group. No significant difference in serum creatinine, blood hemoglobin and serum ALT activity were detected between NHL patients and NHL HCV patients (Table 1)

IV-Serum concentrations of IL-22, IL-32α and IL-34 in the patient groups

A significant increase ($p<0.001$) in the serum concentrations of IL-22, IL-32α and IL-34 was detected in NHL patients with HCV infection in comparison to NHL patients without HCV infection (Fig 1). Of note, HCV infection in NHL patients elicited an increase of 1737-fold in IL-34, while the extents of increase were 16-fold in IL-22 and 7-fold in IL-32α, when compared to NHL patients without HCV infection. Moreover, the extents of correlations between these ILs were 0.678 (IL-22 vs. IL-32α; direct medium), 0.731 (IL-22 vs. IL-34; direct strong) and 0.664 (IL-32α vs. IL-34; direct medium) (Fig 2).

Table 1. Descriptive statistics of NHL patients and NHL patients with HCV

Parameter	NHL	HCV + NHL
Number (N)	26	26
Female N (%)	13 (50%)	11(42.3%)
Male N (%)	13 (50%)	15 (57.7%)
Age (Range, Mean ± SD)	20-73 49.23 ± 16.15	19-81 55.12 ± 12.77
Ann Arbor Stage (N)		
I	26	1
II	-	5
III	-	13
IV	-	7
Hemoglobin (g/dL)	11.81±1.96	11.97±2.05
Platelets count (×10^3/ µL)	222.7±104.5	159.8±81.66
Total WBCs (×10^3/µL)	7.038±5.44	6.962±4.94
Serum ALT (IU/L)	22.23±10.14	44.77±67.70
Serum AST (IU/L)	24.69±8.93	105.6±368.6**
Serum Creatinine (mg/dL)	0.996±0.27	1.058±0.62
Extra Nodal Liver (N)		
No	18	17
Cirrhotic	4	5
Enlarged	4	4
HCV Treatment (N)	26	7
No	0	19
Yes		
B-cell Symptoms (N)	8	9
No	18	17
Yes		
Treatments (N)		
CHOP	4	9
COP	1	3
DA EPOCH	2	1
GDP	2	-
RCHOP	13	8
RCOP	1	3
RDA-EPOCH	2	-
RGDP	1	-
CHOP+VEPSID	-	1
MINI CHOP	-	1

** denotes statistical significance at $p<0.01$ as determined by Mann-Whitney test after Shapiro-Wilk normality test. NHL (non-Hodgkin lymphoma); HCV (hepatitis C virus); ALT (alanine aminotransferase); AST (aspartate aminotransferase); CHOP (Cyclophosphamide, Hydroxydaunorubicin, Oncovin and Prednisone); COP (Cyclophosphamide, Oncovin and Prednisone); DA EPOCH (Dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin); GDP (Gemcitabine, Dexamethasone, and Cisplatin); RCHOP (Rituximab combined with Cyclophosphamide, Hydroxydaunorubicin, Oncovin and Prednisone); RCOP (Rituximab combined with Cyclophosphamide, Oncovin and Prednisone); RDA EPOCH (Rituximab dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin); RGDP (Rituximab combined with Gemcitabine, Dexamethasone, and Cisplatin)

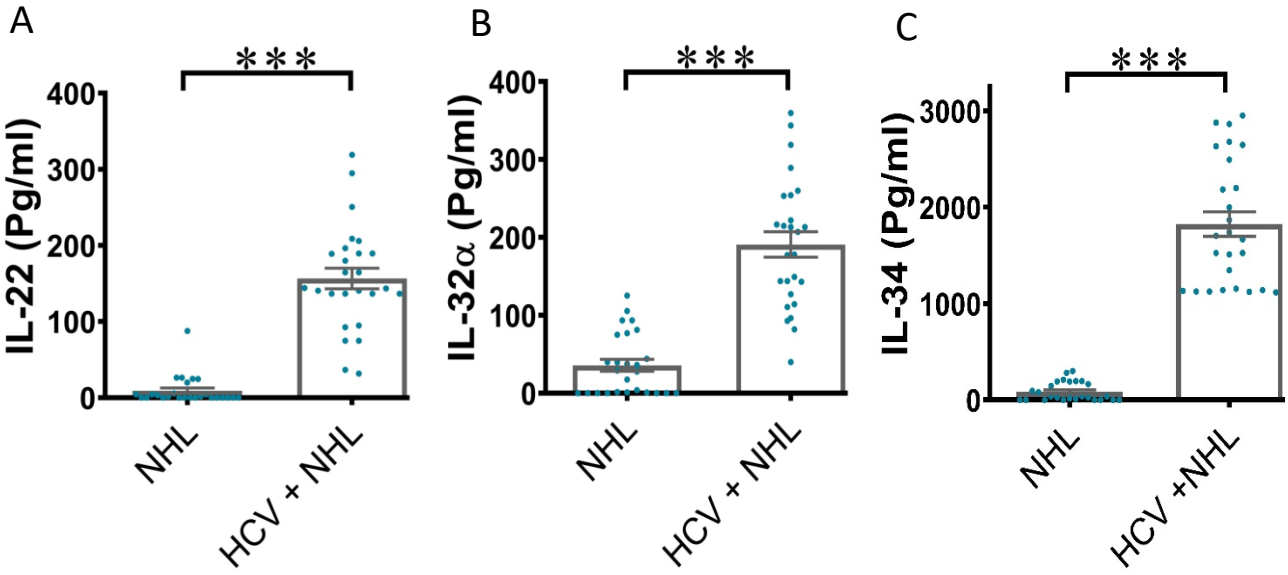

Fig 1. Serum concentrations of interleukin (IL)-22 (A), IL-32α (B) and IL-34 (C) in NHL patients and NHL patients associated with HCV. *** denotes statistical significance at p<0.001 as determined by Mann-Whitney test after Shapiro-Wilk normality test.

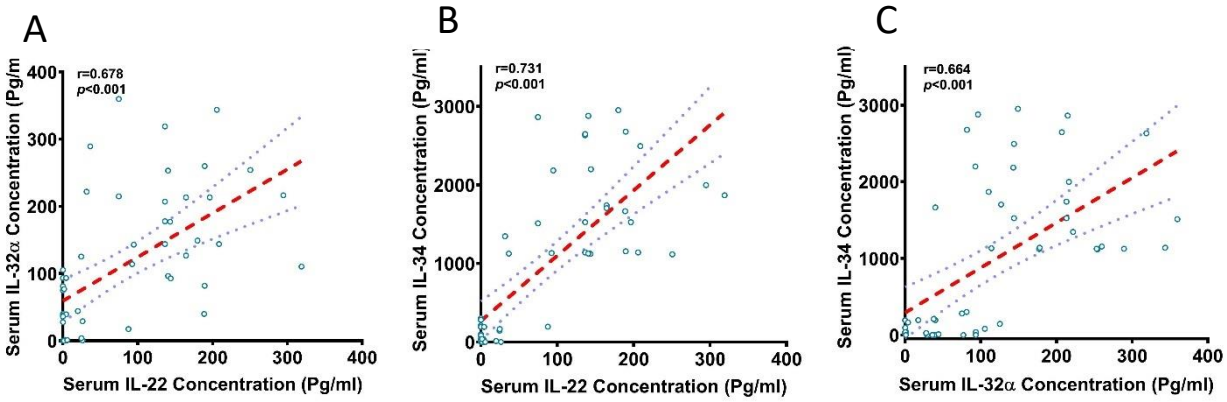

Fig 2. The correlation extents between interleukin (IL)-22 and IL-32α (A), (IL)-22 and IL-34 (B), and IL-32α and IL-34 (C) in NHL patients and NHL patients associated with HCV. Values are expressed as Spearman's correlation coefficient (r). The positive correlation categories were very weak (more than 0 to 0.29); weak (0.3 to 0.49); medium (0.5 to 0.69); and strong (0.7 or more).

Discussion:

B-cell NHL is strongly associated with HCV infections. The most common HCV infection related B-NHL subtypes include MZL and DLBCL lymphomas (3). HCV induces an early immune inflammatory response comprising induction of antiviral and immunoregulatory cytokines that may play a role in NHL progression among HCV patients [3]. On this basis, the aim of the present study was to assess the serum levels of the proinflammatory IL-22, IL-32α and IL-34 in the NHL patients with and without HCV infection.

The present study showed a significant increase in the concentration of serum IL-32α in NHL Patients with HCV, compared to the counterparts without HCV. This observation can be attributed to the role of IL32-α in fibrosis development in chronic HCV by inducing monocyte differentiation, upregulation of other proinflammatory and profibrogenic cytokines, and initiating apoptosis, which might also stimulate fibrogenesis [13, 14]. Moreover, IL-32α can foster the

NHL progression by NF-κB-mediated cytokines and metalloproteinases production. This was consistent with a previous study reported a positive correlation between IL-32α and cancer invasion in lung cancer [15]. Moreover, another study dealing with breast cancer demonstrated a positive link between IL-32α expression and the tumor size, lymph node number, metastasis, and tumor stage [16].

IL-32α has also influenced tumor cell motility, a critical factor in tumor cell invasion and metastasis. In gastric cancer, IL-32α-overexpressing cells displayed significantly higher invasive, metastatic and wound healing capacities, compared with those of control cells. The underlying mechanism of IL-32α-triggered cell invasion was an increase in the expression of IL-8, vascular endothelial growth factor and matrix metalloproteinases (2 and 9), as well as activation of protein kinase B, β-catenin and hypoxia inducible factor-1α [17]. These findings were in line with those of other study, which indicated that the IL-32α-acquired invasive and migration phenotypes of lung cancer were mediated through the co-expression of IL-8 and vascular endothelial growth factor [18].

Regarding IL-34, the serum rise of IL-34 in NHL with HCV infection was consistent with its the role in stimulating the resident macrophages to secret chemokine ligands for recruitment of more monocytes and macrophages, as well as stimulating the release platelet-derived growth factor and transforming growth factor-β1 that drive liver fibrosis [19]. IL-34 also plays an important role in cancer progression due to its capability to recruit tissue associated macrophages, which can drive neo-angiogenesis and metastasis in cancers [20]. Moreover, the IL-34/CSF-1R signaling contributes to extravasation of immune cells and formation of new vessels in a paracrine manner [21]. IL-34 also activates AKT leads to triggering C/EBPβ signaling, thereby augmenting the immunosuppressive activity of tissue associated macrophages in the tumor microenvironment [22].

Accumulating evidence also suggests that IL-34 acts through autocrine and paracrine mechanisms to promote carcinogenesis. In the autocrine pathway, IL-34 interacts with the M-CSF1-R on cancer cells, leading to activation of signaling pathways stimulating cancer cell growth or enhancing their resistance to chemotherapeutic drugs [21]. In the paracrine pathway, IL-34 is produced by neoplastic cells and/or immune cells and triggers the M-CSF1-R signaling in tumor-associated macrophages, leading to extravasation and recruitment of immune-inflammatory cells and formation of new vessels in the tumor area [23].

The present study also demonstrated the rise of the serum IL-22 concentration in NHL patients with HCV in comparison to NHL patients without HCV. This effect can be accounted to the role of IL-22 in promoting liver diseases by enhancing the migration of the immune inflammatory cells into the liver, which can increase T-cells induced hepatocyte injury [24]. This was corroborated with the data that demonstrated the elevated expression of IL-22 in the liver of patients with chronic HBV and HCV infections [25]. It was also hypothesized that IL-22 sustains inflammation and cancer through its actions on epithelial cells through STAT3 phosphorylation, leading to a dysregulated inflammatory response and tissue repair [11].

In conclusion, the present study reported elevation of serum concentrations of the proinflammatory cytokines IL-22, IL-32α and IL-34 in NHL with background of HCV infection. More studies are needed to investigate the detailed signaling pathways of these proinflammatory cytokines to assess their roles in tumor biology and validate these results on a large cohort of patients with NHL to assure the specificity and sensitivity of these parameters.

Conflict of Interest

The authors have nothing to disclose.

Author Contributions

MEH designed the study, constructed the research plan, reviewed the literature and wrote the draft version of the manuscript. MIM, MAG and MAA conducted the biochemical analyses. RA and KF were responsible for patient selection and clinical data collection. MES carried out the statistical analysis of the data, revised and edited the last version of the manuscript.

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