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HBV and HCV Incidence among Non-Hodgkin's Lymphoma Patients in Varna Region (2013-2016)

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Abstract

Several epidemiological studies have discussed the relationship between *HBV* and *HCV* viral infections and the development of *non-Hodgkin's lymphomas (NHL)*. According to WHO, Bulgaria is in the intermediate zone of hepatitis B endemicity, with 3.87% *HBsAg* (+) carriers. The frequency of *HCV* (+) individuals in the general population is lower -1.28%. A national survey from 1999 to 2000 showed a higher proportion of these infections in the Varna region, 5.26% and 1.4%, respectively.

The purpose of the present study was to investigate the incidence of *HBV* and *HCV* infection in patients with different histological variants of *NHL*, based on the presence of *HBsAg, anti-HBc total Ab* and *anti-HCV Ab.*

We analyzed 466 patients with clinical and laboratory diagnosis *NHL*, tested for *HBsAg* and 462 patients, explored for *anti- HCV Ab*. The survey was conducted in the laboratory of clinical virology of "St. Marina" University Hospital, Varna, from 2013 to 2017. Sixty individuals were tested for *anti-HBc total Ab*, ten of which were *HBsAg* positive.

The proportion of *anti-HCV* positive patients with *NHL* was 1.7% (95% CI: 0.8-3.4, n = 8), while *HBsAg* carriers were significantly more - 8.2% (95% CI: 5.8-11.0, n=38) ($p<0.05$). Fifty *HBsAg* (-) patients were tested for *anti-HBc total Ab* assay and positive results were found in 46.0% (95% CI: 31.8-60, n=23) of them.

A higher *HBsAg* and *anti-HCV* positivity was determined in patients with NHL than the average for Bulgaria and Varna region. *HBV* infection dominated in this group compared to *HCV*.

Keywords: *hepatitis B, hepatitis C, non-Hodgkin's lymphomas (NHL)*

Резюме

През последните години въз основа на епидемиологични проучвания се обсъжда връзката между заразеността с *HBV* и *HCV* и развитието на Неходжкинови лимфоми. България се намира в средната зона на хепатит В ендемичност с 3.87% носители на *HBsAg,* докато честота на носителите на *HCV* е по-ниска -1.28%. Национално проучване от периода 1999 г. - 2000 г. показва по-висок относителен дял на тези инфекции във Варненски регион, съответно 5.26% и 1.4%.

Целта на настоящото изследване е да установим заразеността с *HBV* и *HCV* при пациенти с различни видове Неходжкинов лимфом.

Аналазирани са 466 пациенти с клинична и лабораторна диагноза Неходжкинов лимфом, изследвани за *HBsAg*. От тях 462-ма са изследвани за *anti-HCV* в Лаборатория Вирусология на УМБАЛ „Св. Марина"- Варна. За носителство на *anti-Hbc total Ab* са изследвани общо 60 лица, като 10 от тях са *HBsAg* позитивни. Използвани са стандартизирани търговски ELISA тестове.

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Относителният дял на *anti-HCV* положителните пациенти с Неходжкинов лимфом е 1.7% (95%CI:0.8-3.4, n=8), докато носителите на *HBsAg* са сигнификантно повече - 8.2% (95% CI:5.8-11.0, n=38) ($p<0.05$). От отрицателните *HBsAg* пациенти, 50 са изследвани в теста за определяне на *anti-HBc total*. Положителни резултати открихме в 46.0% (95%CI:31.8-60.7, n=23) от случаите.

Установихме по-висока от средната за региона и страната честота на *HBsAg* и *anti-HCV* позитивност при пациентите с Неходжкинови лимфоми. Нашите данни показват по-висока честота на разпространение на *HBV* при тази група пациенти, в сравнение с *HCV*.

Introduction

HBV and *HCV* are hepatotropic viruses with worldwide prevalence. WHO reported that 257 million individuals live with the *HBV.* In Europe, chronic *HBV* infection has been proved in approximately 13 million people, with the prevalence rate varying between 0.1% and 7% (Stasi *et al.,* 2017; WHO, 2017). More than 180 million people in the world are infected with *hepatitis C* (Khaled *et al.,* 2017). Several studies in Europe have shown an unequable spread of *HCV*, ranging from 0.4% to 3.5%, while in some countries (Italy) *HCV* incidence varies even across different regions (ECDC, 2010).

A national multicenter survey (INTERREG II) conducted in Bulgaria from 1999 to 2000 and funded by the European union (EU) established that *HbsAg* (+) were 3.87% and *HCV* positive 1.28% of the total population. According to these results, Bulgaria was categorized in the intermediate zone of *HBV* endemicity and in the low *HCV* endemicity area. Varna and Varna region have higher incidence of these infections - 5.26% and 1.4%, respectively (Theoharov *et al.,* 2015). Chronic *HBV* and *HCV* infections lead to liver dysfunction and complications such as cirrhosis and hepatocellular carcinoma (El-Serag*,* 2015). In the last two decades, various epidemiological studies showed a connection between these two viral infections and the pathogenesis of *NHL*, predominantly B-cell types (Peveling *et al.,* 2013; Li *et al.,* 2018). Genetic and environmental factors were also not excluded (Marcucci *et al.,* 2012; Zignego *et al.,* 2015; Khaled *et al.,* 2017).

The relatedness of *HCV* and *NHL* was supported by the fact that regression of lymphoid disease was established after antiviral therapy (Vallisa *et al.,* 2005). Other authors believe that 8% of *NHL* is probably due to chronic infection (Taborelli *et al.,* 2016). On this basis, three theories have been discussed: 1) continuous external stimulation of lymphocyte receptors by viral antigens with subsequent proliferation; 2) oncogenic effects mediated by intracellular viral proteins during replication in B- cells. 3) the so-called 'hit-and-run' theory based on continuous B-cell damage by transient intracellular virus and mutation in tumor suppressor genes (Peveling *et al.,* 2013).

The mechanisms underlying the *HBV*-induced *NHL* are not so well defined, but are considered to be similar to those of HCV-provoked lymphoma diseases. *HBV* is believed to be able to infect lymphocytes, and integration into the genome of the host cell may result in overexpression of cellular oncogenes or suppression of tumor suppressor genes. The second mechanism recognizes the role of chronic stimulation by viral antigens without obligatory lymphocyte infestation. Last but not least, a multifactorial model, involving other lymphotrophic viruses and a chronic antigenic stimulus underlying autoimmune diseases were suggested (Marcucci *et al.,* 2012).

The purpose of this study was to investigate the incidence of *HBV* and *HCV* infection in patients with different histological variants of *NHL*, based on the presence of *HBsAg*, *anti- HBc total Ab* and *anti-HCV Ab.*

Materials and methods

Study population

We analyzed 466 *NHL* patients, of whom 53.6% (95% CI: 49.0-58.2, n = 250) were male. The mean age was 61 years (SD ± 14.8) (1 year - 90 years). Patients with *B-cell lymphoma* - 89.1% (95% CI:85.9-91.7, n=415) and especially with *Diffuse Large B-Cell Lymphoma (DLBCL)* prevailed (Table 1).

All participants were tested for *HBsAg* and 462 of them for *anti-HCV Ab*. Sixty persons were tested for *anti-HBc total Ab*, 50 of which were *HbsAg* (-) negative.

Methods

Serological method

Serum samples were investigated for hepatic markers using commercially available *ELISA* test kits - *HBsAg* (Surase B-96, General Biologicals Corp.), *anti-HBc total Ab* (Dia.Pro Diagnostic Bioprobes) and *anti-HCV Ab* (Nanbase C-96, V4.0 (General Biologicals Corp.).

Table 1. Characteristic of patients depending on histological variant

NHL-type	All	Proportion (95%CI:)
DLBCL	193	41.4% (36.9-46.0)
FL	56	12.0% (9.2-15.2)
CLL/SLL	159	34.1% (29.8-38.6)
WM	2	0.4% (0.1-1.5)
MCL	3	0.6% (0.1-1.9)
MZL	2	0.4% (0.1-1.5)
unspecified	40	8.6% (6.2-11.5)
T cells lymphoma	11	2.4% (1.2-4.2)

Table 2. Characteristic of patients positive for HBsAg, anti -HBc total Ab and anti –HCV Ab

Characteristic	HBsAg		anti HBc Ab without HBsAg		Anti HCV	
	n/%	n, % positive	n/%	n, % positive	n/%	n, % positive
All	466 (100.0)	38 (8.2)	50 (10.7)	23 (46.0)	462 (99.1)	8 (1.7)
Man	250 (53.6)	23 (9.2)	32 (12.8)	13 (40.6)	248 (99.2)	4 (1.6)
Woman	216 (46.4)	15 (6.9)	18 (8.3)	10 (55.6)	214 (99.1)	4 (1.9)
age						
0-19	5 (1.1)	0	4 (80.0)	1 (25.0)	5 (1.1)	0
20-34	23 (4.9)	1 (4.3)	4 (17.4)	1 (25.0)	23 (4.9)	0
35-49	56 (12.0)	9 (16.1)	4 (7.1)	2 (50.0)	55 (98.2)	3 (5.5)
50-64	161 (34.5)	16 (9.9)	21(13.0)	12 (57.1)	160 (99.4)	2 (1.3)
65+	221 (47.4)	12 (5.4)	17 (7.7)	7 (41.2)	219 (99.1)	3 (1.4)
histological variant						
B cells lymphoma	415 (89.0)	32 (7.7)	42 (10.1)	19 (45.2)	413 (99.5)	6 (1.5)
T cells lymphoma	11 (2.4)	2 (18.2)	4 (36.4)	2 (50.0)	11 (100.0)	1 (9.1)
Unspecified	40 (8.6)	4 (10.0)	4 (10.0)	2 (50.0)	38 (95.0)	1 (2.5)

Real-time PCR

Real-time PCR for quantitative detection of *HCV RNA* was carried out. Nucleic acid extraction was performed according to the manufacturer's protocol with a commercially available test kit MAGREV Viral *DNA / RNA* 96 Extraction kit / Anatolia Gene Bosphore from 500 µl serum in a final volume of 60 µl eluate. Amplification of the isolated nucleic acid was made using a commercially available kit based on the Taq-man principle: *HCV* Quantification Anatolia Geneworks Bosphores with reverse transcription and subsequent amplification step from c*DNA*. Amplification was performed with a Quanti StudioDx *PCR* instrument in a final volume of 25 µl. All reactions were fulfilled in the presence of internal control (IC) to identify possible inhibition of the reaction.

Statistical analyses:

Proportion, confidence intervals, average age, and standard deviation were calculated. Pearson's χ^2 test was used to compare data. Values of $p < 0.05$ were considered statistically significant.

Results

In the recent survey, *HBsAg* positive patients were 8.2% (95% CI: 5.8-11.0, n = 38), aged 57.8 years (SD ± 13.6), and 1.7% determined positive for *anti-HCV Ab* (95% CI: 0.8-3.4, n = 8), aged 59.5 years (SD ± 15.9). The proportion of *HBsAg* (+) patients was significantly higher ($\chi^2 = 20.31$, $p < 0.05$). Based on age distribution, positive results prevailed for both tests - *HbsAg* and *anti-HCV Ab* in the age group between 35 and 49 years old - 16.1% (95% CI: 7.6-28.3), and 5.5% (95% CI: 1.1-15.1), respectively. No statistically significant sex differences were detected. In one patient, both tests were positive (Table 2).

HBsAg was detected to be positive among 7.7% (95% CI: 5.3-10.7) of all of the *B-cell phenotype (DLBCL, FL, CLL/SLL)* cases and even higher - 18.2% (95% CI: 2.3-51.8) in *T-cell phenotype* patients, without statistically significant difference ($x^2 = 1.5998$, $p = 0.206$) (Fig. 1).

Fifty patients of all *HBsAg* negative ones (n = 428) were probed for *anti-HBc total Ab*. Positive results were found in 46.0% (95% CI: 31.8-60.7), of which 19 cases with *B-cell lymphomas*, two patients were undetermined, and two were diagnosed with *T-cell lymphoma* ($p = 0.856$).

Positive for *anti-HCV Ab* were six patients with *B-cell lymphoma* (*DLBCL* - 5, *CLL* - 1), one - with T-cell lymphoma, and one untreated patient ($p = 0.049$) (Fig. 2).

HCV RNA was tested in six of all eight *anti-HCV* (+) patients. The mean viral load in *real-time PCR* was 5.76 lg IU/ml (± 0.54).Two individuals were *HCV RNA* negative.

Discussion

The term *NHL* includes a variety of histologically and biologically different malignancies, originating from the lymphoid system. There are geographical differences regarding morbidity and subtype distribution, as a result of a combination of demographic, ecological and other yet unidentified factors. (Skrabek *et al.,* 2013). In 2016 the newly diagnosed *NHL* cases among the general population in Bulgaria were 4.8 per 100 000 persons. For the city of Varna and Varna region this incidence

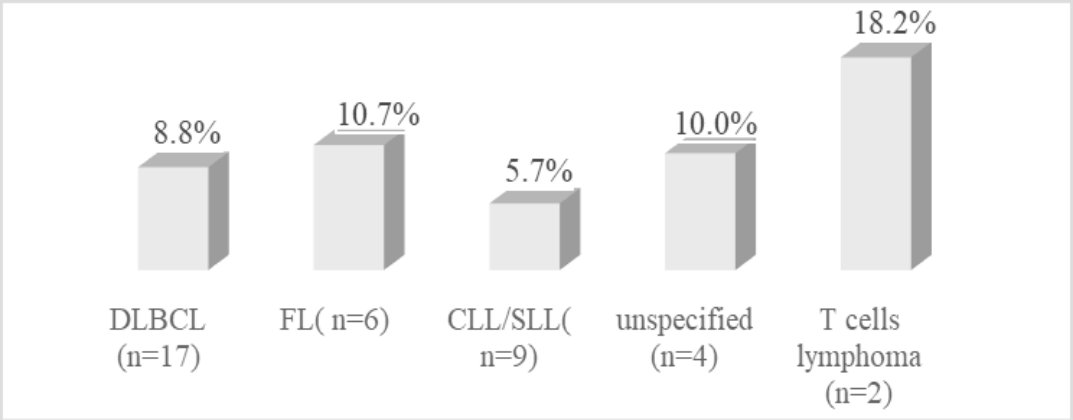

Fig. 1. HBsAg positive patients depending on histological variant

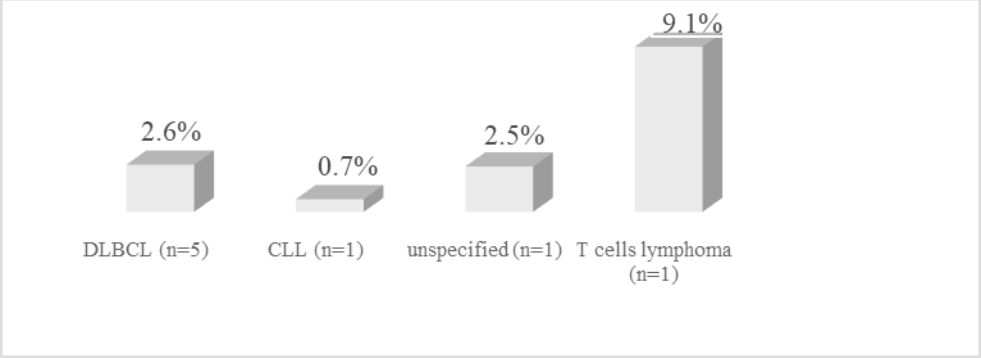

Fig. 2. Anti-HCV positive patients depending on histology and age

rate was 6.8 per 100 000 persons according to official statistics (*NSI*, 2017). Various infections as well as immunosuppression and immune deficiency are considered as risk factors for the development of *NHL*. According to the literature, infectious agents are classified into three groups: 1) viruses that can directly transform lymphocytes (*Epstein Barr Virus, Human herpesvirus 8, Human T lymphotropic virus*); 2) *Human immunodeficiency virus (HIV),* which leads to depletion of CD4+ T lymphocytes and is associated with a high risk of lymphomas in some subtypes; 3) Infectious agents leading to chronic immune stimulation *(HCV, HBV, Helicobacter pylori, Borrelia afzelii, etc.)* (Engels, 2007).

Patients with *Hodgkin's lymphoma (HL)* and *NHL* are often the subject of our studies due to the frequent presence of viral infections in the etiopathogenesis of these diseases, as well as the possibility of reactivation of latent infection as a result of immunosuppressive therapy. In a previous study, we found that the proportion of *EBV DNA* positive patients with *HL* and *NHL* was respectively 40.7% and 15.4% (data not shown).

In this study, we analyzed the incidence of *NHL* patients with *HBV* and *HCV*. According to different studies, *HCV* infection is associated with a 2.5-fold increase in the risk of lymphoma, especially in regions endemic for the viral infection. Data analysis from several regions in the world showed 3.6% *anti-HCV Ab* positive *NHL* patients compared to 2.7% in the control groups (De Sanjose *et al.,* 2008). In a survey conducted in Italy, a significantly higher percentage was reported - 13.8%, compared to 6.4% for the controls (Taborelli *et al.,* 2016). *HCV*-positive patients with lymphomas in Egypt, where the incidence of *HCV* is above 15%, are between 26% and 42.1% (Youssef *et al.,* 2012; Abu-Taleb *et al.,* 2013*;* Khaled *et al.,* 2017). There is an interesting study by Youssef et al. (2012), who found an unusually high percentage of occult *HCV* infection in patients with lymphoproliferative disorders (20%) when compared to healthy volunteers (4%) (Youssef *et al.,* 2012).

Hepatitis C viral infection is not widespread in Bulgaria. In the present survey, we found a higher proportion of *anti-HCV Ab* positive patients with *NHL* when compared to the general population in Bulgaria and Varna, although the differences were not significant ($p = 0.401$ and $p = 0.578$, respectively). In another Bulgarian study for Plovdiv and Plovdiv region (2013), also a higher proportion of *anti-HCV Ab* positive newly diagnosed *NHL* patients (1.84%) was reported of the total population (1.28%) and the population of the region (1.1% are from the 2000 survey). A statistically significant association was recorded for indolent lymphomas (Grudeva-Popova *et al.,* 2013).

Although we found a higher proportion of positive T-cell lymphoma patients, most often *HCV* infection prevailed in individuals with lymphoma B-cell type, and especially in persons with *DLBSL, MZL and lymphoplasmacytic lymphomas (Waldenström's macroglobulinemia)* (De Sanjose *et al.,* 2008; Pozzato *et al.,* 2016). These conclusions were also confirmed by the study of Taborelli *et al.* (2016) with the highest frequency of positive patients with *lymphoplasmocytic lymphoma* (28.6%) and *MZL* (27.3%), tested in *real time PCR*.

The relationship between *NHL* and *hepatitis B* virus infection is less studied. An increased risk was demonstrated in *HBV*-infected individuals with significant heterogeneity with respect to the histological subtype, based on different meta-analyses of a large number of cases from various studies (Li *et al.,* 2018). We found a significantly greater incidence rate of *HBV*, compared to *hepatitis C* infection. Patients with lymphoma had a higher proportion (38/466) when paralleled with *HBsAg* positive individuals, found at national level (448/11597) and at regional level (131/2492), and differences were statistically significant ($p < 0.005$). A higher percentage in this group was recorded in Plovdiv region, Bulgaria - 5.72% (Grudeva-Popova *et al.,* 2013) and in Italy (3.7%) (Taborelli *et al.,* 2016). WHO classified Bulgaria and Italy as zones with intermediate *HBV* endemicity (ECDC, 2010), but in both of our studies, *HBsAg* positivity was more frequent. According to the literature data, *B-cell lymphoma subtypes* (*DLBCL* and *FL*) have a significantly stronger relationship. In countries with an intermediate and high endemicity rate of *HBsAg*, such as countries in Asia and Europe, this relationship was more significant than in countries with low *HBV* prevalence (Li *et al.,* 2018).

In Bulgaria, compulsory immunization against *hepatitis B* virus was introduced in 1992. This may explain the decrease in the number of *HbsAg* (+) individuals in the young age groups. Due to the fact that *NHL* develops in elderly patients, the effect of immunization should be evaluated in the future.

An intriguing association between occult *HBV* infection (OBI) and *NHL* was found. *HBV DNA* was proved to be retained in the liver and/or lymphocytes of the macroorganism for many years or even throughout life (Marcucci *et al.,* 2012). In

these cases, viral reactivation is a very plausible scenario (Rosato *et al.,* 2015). Some authors have reported that reactivation of OBI in this group varies between 6% and 10% (Chen *et al.,* 2008; Rossi *et al.,* 2009; Masarone *et al.,* 2014; Elbedewy *et al.,* 2015) and according to others - 21% - 28% (Hanbali *et al.,* 2009; Francisci *et al.,* 2010; Cheung *et al.,* 2011; Watanabe *et al.,* 2011). A survey among 3922 *HbsAg* (-) individuals in Varna and Varna region was conducted from 2010 to 2015. They were separated into two groups – 2 326 randomly selected persons, and 1 596 patients with various chronic diseases, including liver damages. *Anti-HBc total Ab* (+) results were documented in 8.25% (95% CI: 7.2-9.4, n = 192) of people from the first group. According to the same investigation, for the second group, *anti- HBc total Ab,* only the positive patients were 23.6% (95% CI: 21.6-25.8, n = 377) (Tsaneva-Damyanova *et al.,* 2016). In our study, *anti-HBc total Ab* (+) were a significantly higher proportion - 46.0%. *Real Time PCR* data for defining true occult HBV infection will be the subject of future trials.

Conclusion

Our recent investigation manifested a significantly higher incidence of *HBsAg* positivity in *NHL* patients compared to the average HBV incidence for the country and the region. The proportion of *HCV* infection was not found to be significantly different. *HBV* infection dominated over *HCV*, which was in line with the information found for the general Bulgarian population. We recommend that those individuals, in addition to *HbsAg* serology, should be tested for *anti-HBc total Ab,* and the *anti-HBc total Ab* (+) ones should be verified by *PCR* for occult *HBV* infection. *PCR* should be considered as a basic test for subsequent adequate therapeutic solutions for *HBV* and *HCV* viral infections.

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