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Original Article

Evaluation of the risk of developing hepatocellular carcinoma in chronic Hepatitis C patients receiving antiviral treatment

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Abstract

Introduction: Chronic HC leads to the development of liver cirrhosis (LC) and hepatocellular carcinoma (HCC). The treatment of chronic HC with DAAs reduces mortality from LC and HCC.

The study aimed to investigate the serological markers specific to HCC (PIVKA-II and AFP) in patients with chronic HC before and after DAA treatment.

Methodology: The study involved 35 HCV patients (mean age: 56.23 ± 1.45) divided into two groups. Group 1 included 15 HCV + HCC patients and Group 2 included 20 HCV non-HCC patients.

Results: At the end of treatment all the patients were HCV RNA negative. Three months after the end of antiviral treatment, HCV RNA was undetectable in all patients, while a complete biochemical and virological response was observed in 66.7% of HCV + HCC patients and 85.0% of HCV non-HCC patients. PIVKA-II levels before the initiation of antiviral treatment were high in all patients. At the end of the treatment, in the HCV non-HCC group, normalization of PIVKA-II levels was observed only in 20.0% cases, and in 60.0% of cases 3 months after the treatment. Meanwhile, in patients with HCC and chronic HCV, PIVKA-II levels were within the normal range 3 months after treatment in only 13.3% of patients.

Conclusions: It is necessary to monitor HCV patients with cirrhosis (F4) and severe fibrosis (F3) without HCC, who have high PIVKA-II and AFP levels and/or ALT activity despite obtaining sustained virologic response 3 months after treatment with DAAs.

Key words: Chronic HCV infection; hepatocellular carcinoma; liver cirrhosis; direct-acting antiviral agents.

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Introduction

Liver diseases make up a significant percentage among causes of disability and death worldwide. The rates of sickness are noticeably on the rise, especially in working-age people with chronic liver problems. Intense research of the latter is justified given its social impact. Chronic Hepatitis C (HC) has a special place among liver diseases. Chronic hepatitis C virus (HCV) is unique because it can stay hidden and thus undiagnosed for a long time. Simultaneously, it progresses gradually, leading to the development of liver cirrhosis (LC) and/or hepatocellular carcinoma (HCC) [1-3].

Presently, the antiviral drugs used for treating chronic HC have 95% effectiveness in treating the disease, which leads to a decrease in mortality from LC and HCC. However, the accessibility of diagnosis of chronic HC and treatment remains low. According to WHO data, the number of HCV-infected individuals rises every year. In 2019, for example, it reached 1.5 million with 290,000 lethal cases mainly from LC and/or HCC [4].

The statistics in Armenia have been recorded since 2015. Armenia is considered a country with medium HC prevalence. According to the annual reports of the National Center of Disease Control and Prevention of the Ministry of Health, seropositivity in the adult population is 4%, from which 70% (i.e., 2.8% of the adult population) has chronic HC infection. Amongst those, only 10% are aware of being infected. Thus, the main problem is the low number of informed and diagnosed patients and an even lower number of patients who have started treatment or have been cured. For example, in 2017, in Armenia there were 711

recorded cases of HC, in 2018 there were 1351 new cases, while in 2021 there were 961 new cases (32.6 cases per 100,000).

Several factors have a negative impact on the natural course and the effectiveness of antiviral treatment of HCV. Among such factors are advancing age, male gender, obesity, liver steatosis, insulin resistance, severe fibrosis and LC, accompanying diseases, early menopause, patients' commitment to the treatment [5-10].

HCV is the second most common etiological factor for HCC worldwide and the first in Western Europe, the USA, and Japan. In the case of HCV infection, HCC develops in 1-4% of patients with LC per year, and 13% in patients with Child-Pugh class A cirrhosis after 5 years of observation, which point to the development of primary liver cancer [11].

The risk factors for the development of HCC in HCV infected patients are male gender, patients' age (over 50), high histologic grade, severe fibrosis, HCV 1b genotype. In HCV infected patients, the HCC nodules are separate, small, and have a capsule, as opposed to tumors developed in chronic hepatitis B patients, which have multiple nodules and often have infiltrative growth [12].

For patients with the mentioned risk factors, it is recommended to determine serum alpha-fetoprotein (AFP) levels and conduct abdominal ultrasound imaging every six months for early HCC detection. However, in 40-50% of patients, the levels of AFP do not increase even after the tumor has shown significant growth, and only in 1/5 of patients, it reaches high, diagnosable levels (400 ng/ml) and is considered a negative predictive value. Only in 1/5 of patients, the AFP level correlates with the stage of the disease [13-15].

Currently, three types of AFP have been discovered, of which ACE L3 is mainly present in HCC patients' serums [16].

In the case of HCC detection, ACE L3 sensitivity fluctuates between 45% with 2 cm or smaller tumors and 90% with tumors bigger than 5 cm [17].

The diagnostic value of computer tomography (CT) and magnetic resonance imaging (MRI) is also dependent on the size of the tumor. The precision of MRI is 90% if the tumor is 2 cm or bigger, however, this value decreases to 34% if the tumor is smaller than 1 cm [18].

Although the majority of researchers agree that the diagnostic value of ultrasound is lower than that of CT and MRI [19,20], for HCC screening, ultrasound is used. CT and MRI are not used due to their high cost and cumulative negative risk: X-ray radiation (CT) and the risk of kidney function loss in patients with hepatorenal syndrome caused by iodine contrast use (MRI) [21].

Des-gamma-carboxyprothrombin, also known as PIVKA-II (Protein induced by vitamin K absence or antagonist II), is a relatively new HCC marker. Its level increase is observed in 67% of HCC patients, but only in 8% of patients with small tumor size (< 2 cm). Unlike AFP, PIVKA-II levels do not increase in patients with benign liver diseases, including hepatitis and cirrhosis [22,23]. Nakagawa *et al.* have shown that in HCC diagnostics PIVKA-II sensitivity is 48-62%, and its specificity is 81-98% [24]. PIVKA-II diagnostic value is comparable to AFP. It has been shown that PIVKA-II and AFP are independent of each other and the use of both (PIVKA-II and AFP) markers leads to a significant increase in sensitivity (74.3%) and specificity (87.2%) [25].

Recent studies have demonstrated that within two years after the completion of treatment for chronic HC with direct-acting antiviral agents (DAAs), about 5% of patients can develop HCC [26,27].

Several studies have reported risk factors for HCC after treatment with DAAs. Our initial hypothesis is that if high levels of PIVCA-II and AFP and/or ALT activity persist in chronic HC patients after the standard treatment with DAAs, chronic inflammation in hepatocytes may further lead to the development of HCC.

Based on the above, our work aimed to study the serological markers of HCC (PIVCA-II and AFP) in patients with chronic HC before and after the treatment with DAAs.

Methodology

The study included 35 untreated patients (30 males and 5 females, mean age 56.23 ± 1.45) diagnosed with HCV-related chronic hepatitis. The study participants were selected from infection and gastroenterology-hepatology departments of the medical center based on the diagnosis of hepatitis C. Patients were divided into two groups. The first group (HCV + HCC) included 15 patients diagnosed with HCC along with HCV, all males, mean age 57.06 ± 2.42. The second group (HCV non-HCC) included the remaining 20 patients (15 males and 5 females, mean age 55.20 ± 1.80).

The diagnosis was based on clinical (medical history, clinical examination), instrumental (ultrasonography, liver elastography, CT scan, liver biopsy), and laboratory (serum HCV antibodies, HCV-RNA and liver function tests, AFP, PIVKA-II test) data.

Patients with autoimmune diseases, alcohol abuse, and drug-induced liver injury were excluded from the study. According to the recommended screening strategy, patients with other chronic or acute infectious processes (altered white blood cell count, temperature, urinary tract infection, airway infections, etc.) were also excluded. Patient's plasma des-gamma-carboxyprothrombin levels were determined by sensitive enzyme immunoassay (ARCHITECT PIVKA-II, Japan, cut-off 50.9 mAU/mL) according to the manufacturers' instructions. Informed consent was obtained from all patients. While fasting in the morning, blood samples were drawn from all subjects, and the testing was conducted by Prom-Test laboratories.

There was no need to determine HCV genotype in this study since the treatment was conducted with pangenotypic DAAs. However, according to their medical history, the majority of patients had undergone testing before and in most cases, HCV 1b genotype was present, which is the most prevalent in Armenia.

Liver ultrasound elastography was performed in all patients with the CANON Medical Systems APLIO I900 device. Fibrosis level was expressed in kPa: F2 - mild fibrosis (6.3 - 10.7 kPa), F3 - severe fibrosis (8.1 – 13.5 kPa), and F4 - cirrhosis (18.5 - 30.7 kPa). In the first group, the results showed F3 in 6.7%, F3-F4 in 13.3%, and F4 in 80.0% of patients, while in the second group F2 in 10.0%, F3 in 10.0%, F3-F4 in 15.0%, and F4 in 65.0% of patients. To precisely determine the fibrosis stage, six patients underwent liver biopsy.

Subjects were tested 3 times: before the antiviral therapy, after 12 weeks (end of treatment), and after 24 weeks. After the end of treatment, the patients were tested for HCV RNA with negative results. The overwhelming majority of patients underwent treatment with DAAs under a government program according to international guidelines [28,29] - Sofosbuvir/Velpatasvir, Sofosbuvir/Daclatasvir, 1 tablet per day and/or 1 tablet of each for 12 weeks. All patients with 1b genotype in the stage of cirrhosis underwent treatment with Sofosbuvir/Ledipasvir for 12 weeks [29].

HCC diagnosis was based on serum PIVKA-II and AFP levels, liver ultrasonography, and CT scan data.

Statistical analysis

Statistical analysis of the data was performed using the STATISTICA 6.0 software. After assessing normality of distribution, the results of biochemical examinations were evaluated by the statistical dispersion method using the Student test. The relationship between continuous variables was studied using Spearman's correlation test. As the distribution was normal, a paired sample t-test was used to test the statistical significance of the difference between the values of pre- and post-treatment. Statistical significance was set at $p < 0.05$.

Ethics

This study was approved for conduct by the Ethics Committee of Yerevan State Medical University after M. Heratsi. Informed consent was obtained from each participating patient.

Results

In the first group (HCV + HCC), before starting antiviral therapy, high levels of PIVKA-II, AST and GGT were recorded in 100.0% of patients, ALT in 86.7% of patients and AFP in 73.3% of patients. The overwhelming majority had low PLT (93.3%) and PT% (86.7%) count. After the treatment, PIVKA-II levels remained high in all subjects, while AFP levels increased in 60.0% of patients. The ALT and GGT levels decrease was the most significant—53.3% and 40.0%, respectively. PLT count was unchanged and PT% continued to remain low in 66.7% of the patients. After the third examination (24 months) we recorded high levels of PIVKA-II in 86.7%, AFP in 40.0%, and AST in 80.0% of patients. In this timeframe, a significant normalization of GGT in 73.3% and ALT in 66.7% of patients was recorded, as well as PT% normalization in 40.0% of the cases. PLT count remained low in 73.3% of patients.

The second group (HCV non-HCC) before starting antivirals also showed high PIVKA-II and AST levels, however, GGT levels were high only in 75.0% of the patients. The rest of the comparable indexes were as follows: high ALT in 85.0% and AFP in 50.0% of cases, low PLT (85.0%) and PT (55.0%) count. After the treatment, PIVKA-II levels remained high in 80.0% of the patients, while AFP levels were above normal only in 25.0% of cases. ALT decrease was the most significant, it was above normal only in half of the patients (50.0%), while AST and GGT levels remained high in 80.0% and 60.0% of the cases accordingly. PLT count was low in 60.0% and PT% in 35.0% of patients. After the third examination, it was recorded that PIVKA-II levels remained high at 40.0%, AFP levels remained high only in 15.0% of the cases, however, AST was high in 55.0%. In this timeframe, PT% count in 90.0%, ALT in 85.0%, GGT in 70.0% and PLT in half of the patients reached normal levels.

Following this, we calculated average values of studied indexes in this timeframe, which are presented

in Table 1. The table shows that PIVKA-II values were high in both groups before the treatment and during the study we recorded their decrease, however, only the second group had a statistically significant decrease in values after the treatment ($p < 0.05$) and after 24 weeks ($p < 0.01$) (Table 1).

AFP levels were also high in both groups before the antiviral treatment and decreased during the study, however, this change was not significant.

In both groups, a significant decrease in some liver function tests (Bilirubin Total, Bilirubin Direct, ALP) was recorded only 3 months (24 weeks) after finishing the treatment, and in HCV non-HCC group Bilirubin Direct was significantly ($p < 0.05$) lower compared to the HCV + HCC group. Only in week 24, a decrease in AST levels ($p < 0.001$) was recorded in the first group, while in the second a decrease was recorded after finishing the treatment ($p < 0.001$). The last values of AST in HCV non-HCC group were significantly lower compared to HCV + HCC group. ALT and GGT levels had the same dynamics in both groups, significantly decreasing ($p < 0.05$) immediately after the end of the treatment, which was reaffirmed during the third phase of testing ($p < 0.05 – p < 0.001$). ALB level decrease was most apparent in HCV + HCC group; the average levels significantly increased in both groups ($p < 0.001$) 24 weeks after starting the treatment and in HCV non-HCC patients already after the end of the treatment ($p < 0.05$).

As mentioned earlier, PLT count in the overwhelming majority of patients was low before starting the treatment, more so in HCV + HCC group (79.19 ± 9.41) than in HCV non-HCC group (109.32 ± 13.51) as shown in the table. Despite the rising count of PLT in HCV + HCC patients ($p < 0.05$) in week 24 it was still considerably below (116.81 ± 9.46) the norm. After finishing the treatment, the PLT count was higher in HCV non-HCC group than in HCV + HCC group ($p < 0.05$).

Among coagulation test values, PT% and INR were studied. Before starting the treatment, prothrombin index was low in the majority of patients (86.7%) from the first group, however, after 24 months an increase of PT% ($p < 0.01$) was recorded. In the second group, PT% index was low in half of the patients and significantly increased by the end of the study ($p < 0.001$). With regard to INR, both groups showed high values, considerably higher in HCV + HCC patients, however, in both groups, it reached the norm after 24 weeks ($p < 0.01 – p < 0.001$).

We also studied the correlations between the tested values in different timeframes. In the first group (HCV + HCC), after the treatment we recorded moderate and strong positive correlations between PIVKA-II and

Table 1. The changes of serum values in different groups (HCV + HCC and HCV non-HCC) in different timeframes of the study.

Serum Values (normal distribution)	Statistical values	Research timeframe					
		First group (n = 15) (HCV + HCC)			Second group (n = 20) (HCV nonHCC)		
		Before treatment	After 12 months	After 24 months	Before treatment	After 12 months	After 24 months
PIVKA II mAU/mL (< 50.9)	$\bar{X}$	11237.87	3087.65	816.77	355.67	151.55	56.99
	m	8363.67	1753.78	519.90	85.05	33.26*	°°9.15**
AFP ng/mL (≤ 8.78)	$\bar{X}$	426.84	728.10	34.28	522.60	71.04	20.73
	m	333.39	672.23	20.68	464.22	54.95	12.87
Bil. Total mkmol/L (3.4 – 20.5)	$\bar{X}$	65.25	38.87	27.88	48.20	27.07	15.01
	m	13.69	7.20	7.17*	11.70	5.79	°1.07**
Bil. Direct mkmol/L (0 – 8.6)	$\bar{X}$	38.70	21.52	14.47	27.112	11.46	7.085
	m	10.35	5.56	5.31*	7.76	2.37	+0.94*
ALP IJ/L (40-150)	$\bar{X}$	162.72	123.93	92.10	141.46	108.13	85.69
	m	16.24	12.51	°7.91***	14.34	9.21	6.85**
ALB g/L (35-50)	$\bar{X}$	30.32	33.90	38.85	34.60	37.27	40.32
	m	1.44	1.42	°1.15***	0.94	+0.93*	°0.98***
AST IJ/L (5-34)	$\bar{X}$	152.57	157.07	64.82	149.18	75.1	38.75
	m	17.76	68.65	7.38***	20.61	13.24***	°++5.51***
ALT IJ/L (< 55)	$\bar{X}$	128.32	73.95	52.39	133.21	69.85	39.23
	m	18.18	12.90*	7.12***	16.53	8.85**	°°6.65***
GGT IJ/L (12-64)	$\bar{X}$	214.54	103.02	52.73	197.93	94.05	64.65
	m	39.50	17.83*	°8.90***	39.10	17.53*	14.40**
PLT 10 3/uL (150-400)	$\bar{X}$	79.19	97.92	116.81	109.32	128.14	142.85
	m	9.41	8.86	9.46*	13.51	+12.61	15.62
PT % (70-120)	$\bar{X}$	60.18	70.33	77.05	68.77	77.15	85.69
	m	4.01	3.58	4.44**	3.60	2.96	3.12***
INR (0.8-1.2)	$\bar{X}$	1.78	1.39	1.23	1.48	1.316	1.137
	m	0.11	0.08**	0.05**	0.08	0.06	°0.05***

* - statistical significance $p < 0.05$ compared to values recorded before treatment in the same group; ** - statistical significance $p < 0.01$ compared to values recorded before treatment in the same group; *** - statistical significance $p < 0.001$ compared to values recorded before treatment in the same group; + - statistical significance $p < 0.05$ comparing two groups HCV + HCC and HCV non-HCC in the same timeframe; ++ - statistical significance $p < .01$ comparing two groups HCV + HCC and HCV non-HCC in the same timeframe; o - statistical significance $p < 0.05$ compared to week 12 in the same group; oo - statistical significance $p < 0.01$ compared to week 12 in the same group.

Bilirubin Total ($r_s = 0.61$; $p < 0.05$), Bilirubin Direct ($r_s = 0.73$; $p < 0.05$), ALP ($r_s = 0.66$; $p < 0.05$), ALT ($r_s = 0.84$; $p < 0.05$), inverse correlation with ALB ($r_s = -0.62$; $p < 0.05$), no correlation between PIVKA-II and AFP ($r_s = 0.32$; $p < 0.05$). At the end of the study (week 24), strong positive correlations were recorded between AFP and Bilirubin Total ($r_s = 0.89$; $p < 0.05$), Bilirubin Direct ($r_s = 0.73$; $p < 0.05$) and, again, no correlation was recorded between PIVKA-II and AFP ($r_s = 0.15$; $p < 0.05$).

In the second group (HCV non-HCC) moderate positive correlation between PIVKA-II and AFP was detected at the end of the treatment ($r_s = 0.58$; $p < 0.05$) as well as after 24 weeks ($r_s = 0.56$; $p < 0.05$).

We decided to compare PIVKA-II and AFP values only in F4-stage patients. Interestingly, in the first group (HCV + HCC) (n = 12), the correlation between PIVKA-II and AFP ($r_s = 0.79$; $p < 0.05$) was high after the treatment and was even higher in the last tests t ($r_s = 0.94$; $p < 0.05$). In the second group (HCV nonHCC) (n = 13) the analysis of values showed a moderate positive correlation between PIVKA-II and AFP levels after the treatment ($r_s = 0.60$; $p < 0.05$), however, it became weaker in the last phase ($r_s = 0.54$; $p < 0.05$).

Discussion

The revolution in the treatment of chronic HCV patients continues to this day and relies on the active development of DAAs, many of which are either in clinical trials or are already successfully used in practical medicine, even being used in cases with decompensated LC [30]. LC remains the main risk factor for HCC development [31], and in patients with cirrhosis and active HCV infection, the yearly risk of HCC is 3% [32], the latter being considered the fifth most common and the second deadliest cancer worldwide [33].

However, as noted earlier, there is information on the risk of malignancy even after a successful DAA therapy [26,27]. Even in the case of sustained virologic response, 5.8% of the patients will be diagnosed with liver tumors [34].

The results of our research show that all the participating patients had a virologic response after the antiviral treatment. Along with that, the ALT levels, which are considered the traditional measure of a successful treatment, decreased, and the normalization of liver cell cytolysis values was recorded in half of the patients from both groups. Despite ALT levels remaining above the norm, a statistically significant decrease was recorded in both groups.

Three months after the antiviral treatment, HCV RNA was again not detected in any of the patients, however, a full biochemical and virologic response was recorded only in 66.7% of HCC and chronic HCV patients and in 85.0% of patients with chronic HCV only.

As mentioned, PIVKA-II levels were high in all patients before the treatment. At the end of the treatment in the group with HCV without HCC normal values were detected only in 20.0% of the cases and in 60.0% after 3 months. While in the group with HCC and chronic HCV patients, PIVKA-II levels were normal only in 13.3% of subjects after 3 months. We also recorded an increase in AFP, but the rate of its decrease was considerably quicker than that of PIVKA-II. In patients with chronic HCV and HCC, significant statistical differences between serological markers of HCC (PIVKA-II and AFP) were not recorded before the treatment as well as after the treatment. These are independent diagnostic biomarkers, and this result aligns with those of some international publications [25,35]. However, a high correlation was recorded between PIVKA-II and AFP in HCV + HCC group when comparing data from F4 stage patients after the treatment as well as after 3 months. So PIVKA-II and AFP levels showed a high correlation only in patients with cirrhosis in HCV + HCC group, and a moderate correlation in the HCV non-HCC group.

So, the successful treatment of chronic HCV infection with DAAs reduces but does not eliminate the risk of HCC, since even 3 months after the treatment with sustained virologic response our data show that in 40% of the patients PIVKA-II levels remain above the norm. Therefore, even in the case of obtaining a sustained virologic response, continuous monitoring is necessary not only for patients with cirrhosis (F4) but also in cases of severe fibrosis (F3) [36,37]. It is important to correctly assess HCC development risk factors after DAA therapy, although the strongest factor is, of course, the absence of a sustained virologic response [38-40].

It has been reported that age over 50, male gender, diabetes mellitus, esophageal varices [40,41], and metabolic syndrome [36,42,43] can also be risk factors for the development of HCC. It has also been shown that albumin levels < 3.5 mg/dL and thrombocyte count < 120000/mL, as well as the absence of sustained virologic response, are independently tied to a higher risk of HCC development [44]. It should also be noted that chronic inflammation can stimulate the neoplasm of hepatocytes and the development of malignant clones [45].

One of the limitations of this study was that the number of laboratory-instrumental examinations was based on only one medical institution in Yerevan. other medical institutions of regions were not included. Therefore, the results may not represent the situation across the country. It should also be noted that the data have been obtained from a small number of subjects, and a larger study and continuous monitoring are needed to confirm the results.

Conclusions

Therefore, we highlight the need for monitoring of HCV patients with cirrhosis (F4) and severe fibrosis (F3) but without HCC, who have high PIVKA-II and AFP levels and/or ALT activity despite obtaining sustained virologic response 3 months after treatment with DAAs and who also have hypoalbuminemia, thrombocytopenia, hyperbilirubinemia, and coagulopathy.

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Authors' Contributions

All authors have significantly contributed to the work reported in the article. Sona Sargsyan: conceptualization, methodology, data curation, investigation, software, formal analysis, writing - original draft, Hripsime Magdesieva, Tsoghik Navoyan, Aregnaz Mkhitaryan, Lusine Atoyan, Violeta Sargsyan, Hayk Harutyunyan, Vahe Azatyan, Armine Minasyan - contribution to data curation and resources. Naira Gyulazyan - conceptualization, writing - review and editing, project administration, supervision.

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