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CONTENTS

CHARACTERISTICS OF BIOCHEMICAL VALUES IN PATIENTS WITH HCV INFECTION BEFORE CONDUCTING ANTIVIRAL THERAPY DEPENDING ON VITAMIN D LEVEL

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Annotation. *The study involved 100 patients with chronic hepatitis C (HCV) who had not received antiviral therapy before. Characterization of biochemical values before antiviral therapy was performed depending on the level of vitamin D. Patients with HCV were divided into 2 groups depending on the level of vitamin D: group 1 – HCV patients with normal levels of vitamin D, group 2 – HCV patients with decreased level (deficiency and insufficiency) of vitamin D. A liver-synthesized vitamin D metabolite was defined in the blood serum – 25-hydroxycalciferol (25(OH)D), which today is an indicator of vitamin D level adequacy in the human body. To verify the diagnosis of vitamin D deficiency, a classification (M.F. Holick, 2011), adopted by the International Institute of Medicine and the Committee of Endocrinologists, was used.*

The study results showed that when comparing the absolute biochemistry complex values, a statistically significant difference in the level of ALT was revealed, which was confirmed by means of frequency analysis of biochemical disorders (p = 0.02). There were no differences in medians among other values.

Key words: *chronic hepatitis C, vitamin D, vitamin D deficiency.*

Introduction. Vitamin D deficiency affects nearly one billion people worldwide [1]. In addition to its major involvement in bone metabolism, maintenance of enteral absorption of calcium, magnesium, phosphate, iron, and zinc. Vitamin D is responsible for important extraskeletal effects involved in many biological processes. In addition to insufficient exposure to the sun, seasonality, habitat, diet, and degree of skin pigmentation that affect the bioavailability of vitamin D, hepatitis C and B that are the main causes of liver cirrhosis and hepatocellular carcinoma (HCC), may also contribute to vitamin D deficiency as it is known from scientific sources. Low blood serum vitamin D level is associated with many human diseases [2, 3] and is often observed in chronic liver diseases. Reducing the content of vitamin D contributes to the progression of chronic hepatitis B [4, 5] and chronic hepatitis C [6, 7]. There are data on the effect of vitamin D on mortality in chronic liver pathologies, including cirrhosis and hepatocellular carcinoma [8]. The relationship of low levels of vitamin D with the development of liver failure supports the thesis of using a mediator as a prognostic marker in patients with liver cirrhosis [9]. Scientists claim that the level of 25-hydroxyvitamin D3 inhibits the replication of hepatitis C virus [10].

Summarizing the mentioned above, we can conclude that vitamin D deficiency in liver pathologies is associated with decreased intake and intestinal absorption of this vitamin, reduced sun exposure and reduced transport protein content. In addition, vitamin D hydroxylation in liver is impaired in liver diseases, which leads to decreased production of active hormone forms. That is why an in-depth study of the role of vitamin

D metabolism disorders in the pathogenesis of HCV infection remains an urgent question in modern hepatology.

Materials and methods. The study involved 100 patients with chronic viral hepatitis C with HCV genotype 1, 2 or 3, with mean age of 48.78 ± 10.06. 49 of 100 patients were men (49.0%), and 51 were women (51.0%). Patients were listed in the Dnipropetrovsk region register of patients with chronic hepatitis. They had not received antiviral therapy before. Examination of patients was performed according to clinical protocols (according to the Order of the Ministry of Health of Ukraine dated July 7, 2016 No. 729 "On approval and implementation of medical and technological documents for standardization of medical care in viral hepatitis C") and bioethical standards. All patients underwent an objective clinical examination, as well as a set of laboratory and instrumental tests provided for by the relevant protocols of the Ministry of Health of Ukraine (complete blood count and clinical urinalysis, as well as blood biochemistry tests). All these tests were performed according to the common clinical practice methods [11].The etiological diagnosis verification was performed by detecting HCV serological markers in patient's blood and RNA-HCV by means of PCR using the CFX96 test system (BioRad, USA; Vektor-Best-Ukraine) with real-time amplification detection at the NucliSENSeasyMAG RNA / DNA extraction automatic workstation and the Amplicor HCV test, v2.0 system (Roche Molecular Systems, California). Determination of alpha-fetoprotein (AFP) was performed using immunochemical electrochemiluminescence immunoassay (ECLIA) and sandwich principle on the analyzer and Cobas 6000 / Cobas 8000 test systems (Roche Diagnostics, Switzerland). Determination of 25(OH)D was performed using electrochemiluminescence assay method on the Eclia apparatus (Roche Diagnostics, Switzerland) using the analyzer and Cobas 6000 / Cobas 8000 test systems (Roche Diagnostics, Switzerland) in an external laboratory in Dnipro. A liver-synthesized vitamin D metabolite was defined in the blood serum – 25-hydroxycalciferol (25(OH) D), which today is an indicator of vitamin D level adequacy in the human body. To verify the diagnosis of vitamin D deficiency, a classification (M.F. Holick, 2011), adopted by the International Institute of Medicine and the Committee of Endocrinologists, was used. According to this classification, the normal level of 25(OH)D in blood serum is 30-85 ng/ml; the 25-hydroxycalciferol blood level of 29-20 ng/ml is considered to be the vitamin insufficiency, and the value less than 20 ng/ml corresponds to vitamin D deficiency [13]. The laboratory reference values were identical for adults (aged 18 and older).

Statistical data processing was performed using Microsoft Office® and R® software packages [14]. The type of data distribution was determined using the Shapiro-Wilk test. Most of the data had a non-parametric type of distribution, so the quantitative indicators were presented as a median (Me) (1 (Q1) and 3 (Q3) quartiles), and Mann-Whitney(U) test was used when comparing them. Qualitative indicators were presented as n (%); their comparisons were performed using Pearson's $\chi 2$ criterion. The critical level of the p value in statistical hypotheses testing was assumed to be < 0.05.

The study was performed within the framework of the Department research work "Epigenetic Factors of Diseases Associated with Persistent Infections in Children and

Adults" (state registration number 0117U004785).

Results and discussion. The liver inflammation markers were determined by the activity of indicator enzymes (alanine aminotransferase, ALT, and aspartate aminotransferase, AST).

The analysis of inflammation markers (ALT and AST) and other biochemical values, as well as the frequency analysis of biochemical disorders in patients with CHC (depending on the blood level of vitamin D) was performed. The data are presented in Tables 1 and 2.

Table 1

Biochemistry values in CHC patients depending on blood serum vitamin D level

Value, units of measurement (Me [IQR: Q25; Q75]	Group 1 HCV patients with normal vitamin D level (n = 18)	Group 2 HCV patients with vitamin D deficiency and vitamin D insufficiency (n = 82)	p
Total protein, g/L	76.0 [71.0;78.0]	75.0 [66.2;78.0]	0.59
Albumin, g/L	47.0 [44.0;53.2]	45.5 [39.0;50.0]	0.25
ALT, U/L	42.0 [36.0;96.0]	36.0 [18.0;60.0]	0.03
AST, U/L	42.0 [25.5;54.0]	30.0 [24.0;48.0]	0.34
Totalbilirubin, μmol/L	16.0 [11.2;19.2]	16.0 [15.0;22.0]	0.37
Alkalinephosphatase, U/L	90.5 [64.2;127.5]	86.0 [71.0;137.8]	0.71
Alpha-fetoprotein, IU/ml	2.9 [1.9;3.1]	2.9 [1.9;4.2]	0.34
Blood glucose, mmol/L	5.2 [4.7;5.5]	5.3 [4.5;5.5]	0.32
VitaminD, ng/ml	39.0 [37.5;44.3]	19.0 [15.6;23.9]	<0.001

Alanine aminotransferase: ALT values were increased in most patients ofgroup 1 (with normal vitamin D level) – 83.3%, and in half of patients of group 2 (with vitamin D status violation) – 53.7%; the ratio is 1.55 : 1 ($p < 0.05$). The increase level was negligible and amounted to 1.7-1.9 of the maximum standard value (54-60 U/L).

Aspartate aminotransferase: A pattern similar to ALT was observed: AST values were increased in most patients of group 1 (with normal vitamin D level) – 61.1%, and in half of patients of group 2 (with vitamin D status violation) – 47.6%; the ratio is 1.2 : 1 ($p > 0.05$). The increase level was negligible and amounted to 1.5-1.7 of the maximum standard value (48-54 U/L).

Thus, in group 1 patients (with normal vitamin D level), an increase in indicator enzymes was observed more often than in group 2 patients (with vitamin D deficiency), which is specific for both ALT and AST. Probably, this can be explained by the fact that the increase in transaminase activity is explained by the decrease in the expression of enzymes involved in vitamin D hydroxylation of due to necroinflammatory changes in

chronic viral hepatitis C [15].

The ALT and AST increase level median did not exceed 3 standard values among patients in both groups, which corresponds to the minimum degree of activity of chronic hepatitis, and did not depend on the vitamin D level. As we know, chronic viral hepatitis C is characterized by minimal activity of cytolytic processes in the liver. The presented data confirm this fact.

Table 2

Frequency analysis of biochemical disorders in CHC patients depending on blood serum vitamin D level

Value, units of measurement, n (%)	Group 1 HCV patients with normal vitamin D level (n = 18)		Group 2 HCV patients with vitaminD deficiency and vitamin D insufficiency (n = 82)		p
	Standard value	Value change	Standard value	Value change	
Total protein <65 g/L	15 (83.3)	3(16.7)	66(80.5)	16(19.5)	0.78
Albumin < 40, g/L	15 (83.3)	3(16.7)	58 (70.7)	24(29.3)	0.28
ALT > 32, U/L	3 (16.7)	15(83.3)	38 (46.3)	44(53.7)	0.02
AST > 32 U/L	7 (38.9)	11(61.1)	43 (52.4)	39(47.6)	0.30
Totalbilirubin > 20 μmol/L	15 (83.3)	3(16.7)	60 (73.2)	22(26.8)	0.37
Bloodglucose > 6, 4, mmol/L	18 (100.0)	0 (0.0)	80 (97.6)	2 (2.4)	0.50
Alkalinephosphatase >129 с/104 ж, U/L	14 (77.8)	4(22.2)	53 (64.6)	29(35.4)	0.28
Alpha-fetoprotein > 7 IU/ml	10 (100.0)	0 (0.0)	35(83.6%)	6(14.6)	0.19

Protein synthesis liver function. The level of total protein in most patients, regardless of vitamin D status, was normal (group 1 – 83.3%; group 2 – 80.5%). The other patients had minimal hypoproteinemia of at least 60 g/L.

Therefore, the analysis showed that impaired vitamin D metabolism was not associated with impaired total protein level.

The albumin level was decreased in a small number of group 1 patients with HCV: 3 patients (16.7%) versus 24 (29.3%) group 2 patients with vitamin D deficiency. Although there is no significant difference in the registration frequency between the groups, it shall be noted that hypoalbuminemia in patients with vitamin D deficiency was 1.7 times more frequent than in patients with normal vitamin D level.

Bilirubin level impairment. Based on the results of evaluation of total bilirubin level in pre-treatment groups, the analysis showed the following: the indicators were at the same level within the conventional values. In both groups of patients with CHC, regardless of vitamin D status, a normal level of total bilirubin was observed in the

majority of patients; group 1 – 83.3%; group 2 – 73.2%. A few patients in both groups had an increased total bilirubin level (up to 32 μmol/L). Clinically, this was not accompanied by jaundice syndrome.

Therefore, we have found no association of vitamin D level with a pigment metabolism disorder.

Excretory liver function was determined by the activity of alkaline phosphatase (ALP). In group 1 patients, an increased ALP activity was observed in 4 patients (22.2%). In the second group, an increased ALP activity was observed more often than in the first group: namely, in more than a third of people – 35.4%. The level of alkaline phosphatase increase was low and did not exceed 3 standard values.

Summarizing the performed analysis data, we can say that excretory biliary syndrome disorders were infrequently observed in patients with CHC. A slight increase in the incidence of alkaline phosphatase activity in group 2 patients with vitamin D deficiency, in our opinion, is associated with a higher incidence of more severe forms of fibrosis, F3-F4, which leads to a significant impairment of liver architectonics and liver cholestasis.

Alpha-fetoprotein level. Since alpha-fetoprotein level may increase (> 7 IU/ml) in chronic liver lesions and in hepatocellular carcinoma, patients were also examined for alpha alpha-fetoprotein level. Alpha-fetoprotein level was moderately increased only in 6 patients (14.6%) of group 2, who had vitamin D deficiency. In patients with CHC with normal vitamin D level, there was no increase in alpha-fetoprotein level.

Blood glucose. The blood glucose level was not increased in any of the group 1 patients (with normal vitamin D level), and in group 2 (with vitamin D deficiency), the blood glucose level increase was observed in 2 patients (2.4%).

Conclusions. A comparative analysis of ALT and AST biochemical correlates depending on the nature of impairments of vitamin D level in patients with chronic viral hepatitis C showed that a more significant frequency of increase in values of liver inflammatory enzymes was observed in patients with normal levels of vitamin D. Upon comparison of absolute biochemistry complex values, a statistically significant difference in the level of ALT was revealed, which was confirmed by means of frequency analysis of biochemical disorders ($p = 0.02$). In both groups, no regularities were detected between the features of vitamin D level and biochemistry values.

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