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IFLUENCE OF SEASONITY ON VITAMIN D LEVEL IN PATIENTS WITH HCV INFECTION AND HEALTHY PEOPLE

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Ключевые слова: *хронический вирусный гепатит С, витамин D, сезонный фактор*

Abstract. Ifluence of seasonity on vitamin D level in patients with HCV infection and healthy people. Nikolaychuk M.A., Shostakovych-Koretskaya L.R., Budayeva I.V., Biletska S.V. *According to WHO, about 150-200 million people are currently infected with the HCV virus worldwide. Recently, in the professional literature, the number of publications on the role of vitamin D in patients with viral hepatitis C has increased as vitamin D metabolism occurs with the participation of the liver and its deficiency is associated with an increased risk of infectious diseases. The aim of this study was to investigate the effect of seasonal factor on vitamin D (25 hydroxycalciferol) levels* *in patients with chronic viral hepatitis C and healthy subjects. The study involved 100 patients in the registry of patients with chronic viral hepatitis in the Dnipropetrovsk region. The prevalence and deficiency of vitamin D in patients with chronic viral hepatitis C and conditionally healthy subjects at different times of the year were determined, which showed the presence or absence of a seasonal effect on serum 25(OH)D level. Patients were divided into two groups, depending on the time of the year (autumn-winter and spring-summer), in which the level of 25 (OH) D was determined. The serum was metabolised by vitamin D, which is synthesized by the liver – 25 hydroxycalciferol (25 (OH) D), an indicator of the supply of vitamin D to the human body. Vitamin D levels were evaluated according to the M.F. Holick classification. According to the level of vitamin D patients were divided into 3 groups (patients with normal level, insufficient (suboptimal) level and vitamin D deficiency). The results of the study showed no effect of seasonal factor on the level of 25 (OH) D in the serum of patients with chronic viral hepatitis C. Vitamin D levels are controlled by the time of the year: in spring and summer this indicator is normal, in autumn and winter – seasonal decrease in vitamin D.*

Реферат. Влияние сезонности на уровень витамина D среди больных хроническим гепатитом С и здоровых. Николайчук М.А., Шостакович-Корецкая Л.Р., Будаева И.В., Белецкая С.В. *По данным ВОЗ, вирусом гепатита С в мире инфицировано сейчас около 150-200 млн человек. В последнее время в профессиональной литературе возросло количество публикаций, посвященных роли витамина D у больных вирусным гепатитом С, поскольку метаболизм витамина D происходит с участием печени, а его дефицит ассоциируется с повышением риска развития инфекционных заболеваний. Целью работы было изучить влияние сезонного фактора на уровень витамина D (25 гидроксикальциферол) у больных хроническим вирусным гепатитом С и здоровых лиц. В исследовании принимало участие 100 пациентов, находившихся в реестре больных хроническим вирусным гепатитом в Днепропетровском регионе. Проведено определение распространенности дефицита и недостаточности витамина D у больных с хроническим вирусным гепатитом С и условно здоровых лиц в разное время года, которое показывало наличие или отсутствие влияния сезонного фактора на уровень 25 (ОН) D в сыворотке крови. Пациенты были разделены на две группы в зависимости от времени года (осень-зима и весна-лето), в которых проводили определение уровня 25(OH)D. В сыворотке крови определялась основная циркулирующая форма витамина D – 25-гидроксивитамин D(25(OH)D). Оценка уровня витамина D проводилась согласно классификации M.F. Holick. Пациенты были распределены в зависимости от уровня витамина D на 3 группы (пациенты с нормальным уровнем, недостаточным (субоптимальным) уровнем и дефицитом витамина D). Результаты проведенного исследования не выявили наличия влияния сезонного фактора на уровень 25 (ОН) D в сыворотке крови у пациентов с хроническим вирусным гепатитом С. У здоровых лиц уровень витамина D контролируется временем года: весной и летом этот показатель в норме, осенью и зимой наблюдается сезонное снижение витамина D до субоптимального уровня у половины больных.*

Given that the metabolism of vitamin D occurs with the participation of the liver, today in the professional literature the part of research on the role of vitamin D in the pathogenetic mechanisms of liver progression has increased, which indicates the relevance of modern infectology.

Previous studies have reported that patients with chronic hepatitis C (CHC) have a high percentage of vitamin D deficiency as compared to healthy individuals, possibly due to impaired vitamin D metabolism in the hepatobiliary system caused by liver damage [1].

Vitamin D is important among the vitamins necessary for the human body, as it participates in the regulation of metabolic processes occurring in the hepatobiliary system and affects the activity of biochemical processes in liver pathology [5]. The photobiological source of vitamin D in the human body is due to the synthesis of this vitamin in the skin mainly after insolation, in particular as a result of exposure to ultraviolet rays of the spectrum. In humans, this provides from 80% to 90% of the body's needs. Factors that can reduce the formation of vitamin D in human skin include place of residence, time of day (morning and evening), seasonality (winter), air pollution, cloudiness, melanin content in the skin, use of sunscreen, volume of clothing covering the body. Meanwhile, the body's own synthesis of vitamin D can be achieved simply by being outdoors for 20 minutes in outdoor clothing, but in recent years there has been a decrease in the rate of synthesis of endogenous vitamin D. One of the risk factors for vitamin D deficiency in Ukraine is climate. The geographical location of our country is characterized by short summers, which causes an insufficient level of insolation of the population.

A scientific research on the study of the level of 25 (OH) vitamin D in the adult population of different regions of Ukraine (V.V. Povoroznyuk et al., 2011) showed that the level of 25 (OH) D in the serum reaches a maximum in August, although it is in June that the longest daytime of the day, and UV irradiation has the most intense effect at this time.

Seasonality of outbreaks of infectious diseases indicates that environmental conditions have a significant impact on the risk of disease. One of the main environmental factors that can affect this is solar radiation, which acts primarily through ultraviolet radiation (UV), and further control of vitamin D metabolism [2].

The aim of the reseaarch was to study the level of vitamin D (25 (OH) D) depending on the seasonal factor in patients with chronic viral hepatitis C and healthy individuals.

MATERIALS AND METHODS OF RESEARCH

The study involved 100 patients with CHC who were on the register of patients with chronic hepatitis in the Dnipropetrovsk region and had not received antiviral treatment before. Among patients there was such distribution on a genotype of a virus of a hepatitis C (HCV): in n=58 (58%) – 1 genotype was defined, in n=37 (37%) – 3 genotype, in n=5 (5%) patients – 2 genotype. The fourth genotype of VHC was not registered among our patients. In 50% (n=50) high viral load (more than 800000 Ul/ml) was observed, in 50% (n=50) viral load was determined less than 800000 Ul/ml. Analysis of the stage of CHC fibrosis showed the following distribution: 32% (n=32) were diagnosed with the first stage of fibrosis, 30% (n=30) – the second, 26% (n=26) patients – the third, and 12% n=12) patients had the fourth stage of fibrosis.

Diagnosis of chronic viral hepatitis C was carried out in accordance with the instructions on diagnosis, clinical classification and treatment of these diseases in accordance with the order No. 729 from 18.07.2016 "On approval and implementation of medical and technological documents for standardization of medical care for viral hepatitis C". The etiological verification of the diagnosis was confirmed by detecting in the serum of patients of serological markers of HCV and RNA-HCV by PCR using test systems "CFX96" (BioRad, USA); "Vector-Best-Ukraine" with detection of amplification products in "real time" at the automatic station for RNA/DNA isolation "NucliSENS easyMAG" and the system "Amplicor HCV test, v2.0" (Roche Molecular Systems, California). The virus genotype was determined in real time using an analyzer and a test system with the detection of Rotor-Gene amplification products (Corbett Research, Australia); AmpliSens (CIS). Assessment of the stage of fibrosis was performed using a non-invasive assessment of the risk of liver disease – BioPredictive (France), which corresponds to the assessment of the degree of fibrosis on the METAVIR scale (compression elastography of the liver and/or Fibrotest™). Determination of 25 (OH) D was performed using the electrochemiluminescent method on the Eclia apparatus (Roche Diagnostics, Switzerland) using an analyzer and test systems Sobas 6000 / Sobas 8000, Roche Diagnostics (Switzerland) in an independent laboratory in Dnipro. The main circulating form of vitamin D – 25 hydroxyvitamin D (25 (OH) D) was determined in blood serum [7]. The diagnosis of vitamin D insufficiency and deficiency was established according to the latest classification (M.F. Holick, 2011), adopted by the International Institute of Medicine and the Committee of Endocrinologists. According to this classification, the level of 25 (OH) D in the serum from 30-85 ng/ml corresponds to the normal content, the level of 25-hydroxycalciferol – from 29-20 ng/ml in the blood is considered suboptimal vitamin content, less than 20 ng/ml corresponds to vitamin D deficiency [3]. Laboratory reference values were identical: for adults (18 years and older): deficiency: <20.0 ng/ml; suboptimal content: 20.0 – <30.0 ng/ml; normal level (norm): ≥30.0 ng/ml.

The control group included 30 healthy individuals. The selection of patients in this group was carried out purposefully, the main condition of which was the absence of any chronic or acute diseases that could cause a disturbance of vitamin D metabolism.

The electronic database was created on a personal computer using Microsoft Excel (Office Home Business 2KB4Y-6H9DB-BM47K-749PV-PG3KT).

Processing, data analysis was performed using Libre Office and R software packages [6, 4]. For statistical processing of research materials, methods of descriptive and analytical biostatistics were used: verification of the normality of the distribution of quantitative traits according to the Shapiro-Wilk criterion (SW-W). Given that most indicators had a type of distribution other than parametric, quantitative features were presented in the form of median and interquartile range (25%; 75%). The reliability of the difference in medians between the two groups was assessed using the Mann-Whitney test, and in the case of multiple comparisons, by the Kruskall-Wallis test. Qualitative indicators are presented in the form of n (%), and their comparison was performed using the Pearson Chi-square ($\chi 2$) criterion, without the Yates correction for continuity. The effect of seasonality on the probability of developing vitamin D deficiency was assessed by calculating the odds ratio. The critical level of p at <0.05 was taken in testing statistical hypotheses.

The work is a fragment of the planned research work of the department "Immunogenetic predictors of diseases associated with latent infections in adults and children" (state registration number 0115U001214) and "Epigenetic factors of diseases associated with persistent infections in children and adults" (State registration number 0117U004785).

RESULTS AND DISCUSSION

The effect of seasonality on the level of 25 (OH) D in the serum of patients with chronic viral hepatitis C and healthy people in the control group was determined. Patients were divided into two groups depending on the time of the year - autumn-winter and spring-summer, in which the level of 25 (OH) D in the serum was determined.

When studying the mean (Me) level of serum 25 (OH) D in patients with chronic viral hepatitis C and healthy ones, analysis of serum 25 (OH) D showed that the median level of serum 25 (OH) D in patients with HCV was 21.9 (Q1=16.5; Q3=29.0), which corresponds to vitamin D deficiency, and in the control group – 33.1 ng/ml (Q1=27.0; Q3=38,08), which corresponds to the normal content of vitamin D.

When comparing the data of the group of patients with chronic viral hepatitis C and healthy individuals of the control group, it was found that the Mann-Whitney test level of 25 (OH) D in the serum of patients with chronic viral hepatitis C was significantly lower than in patients with chronic viral hepatitis C (p<0.001).

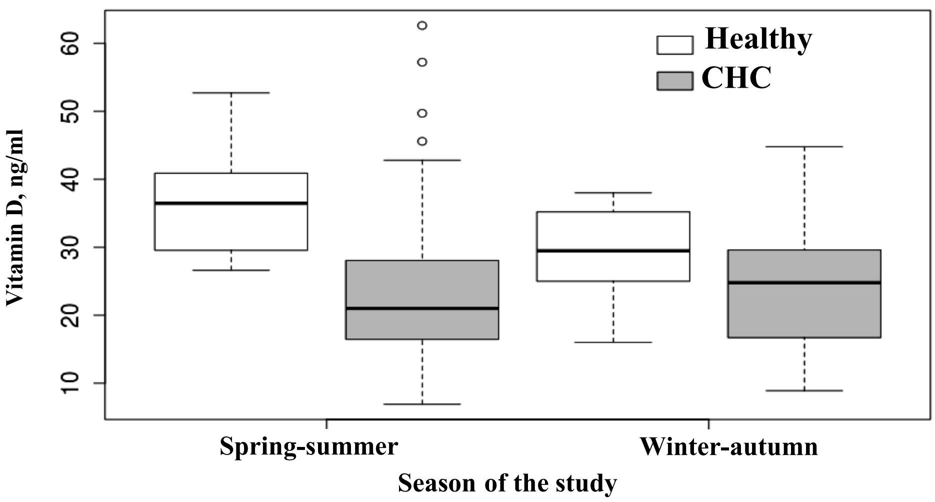
Analysis of the data of the influence of seasonal factor on the level of 25 (OH) D in the serum of patients with chronic viral hepatitis C and in the group of healthy people is presented in the Table 1 and Figure.

Table 1

Serum level of 25 (OH) D in patients with chronic viral hepatitis C and in the group of healthy depending on seasonality

Season	Control group (n=30)	Patients with CHC (n=100)	p_{1-2}
Autumn-winter period (a-w)	**29.5 (25.5; 34.6)**	**24.8 (16.7; 29.6)**	**0.06**
Spring-summer period (s-s)	**36.4 (30.7; 39.9)**	**21.0 (16.4; 28.0)**	**<0.001**
p (a-w) (s-s)	**0.015**	**0.31**	

When analyzing the table of the influence of seasonality on the level of 25 (OH) D in the serum of healthy individuals, it was found that the spring-summer period is the most favorable for the normal metabolism of vitamin D in healthy people. The level of this indicator was normal in the control group at this time of the year. However, in autumn and winter in healthy people there was a relative decrease in the level of 25 (OH) D in the serum to the suboptimal level, but it remained at its upper limit (P (a-w) - (s-s)=0.015).



The content of vitamin D Me (IQR) in patients with CHC and in healthy people depending on the season

A similar analysis of the influence of seasonality in the group of patients with CHC showed the presence of insufficiency of the level of 25 (OH) D in the serum regardless of the season (P (a-w) - (s-s)=0.31). The content of vitamin D approached the lower limit of suboptimal level. This may indicate a negative effect of CHC on vitamin D metabolism.

Frequency analysis of the level of vitamin D in the group of patients with CHC depending on the season showed that regardless of the season in most patients there is a insufficiency or deficiency of vitamin D. The data are presented in Table 2.

As can be seen from the Table, the seasonality factor has no effect on vitamin D metabolism in CHC, and the level of vitamin D will depend on the presence or absence of the disease. The calculation of the odds ratio confirmed the lack of relationship (odds ratio =1.22, confidence interval 0.38-3.90).

Table 2

Analysis of vitamin D levels in the group of patients with CHC depending on the season

Vitamin D-status	Patients with chronic viral hepatitis C n=100		p
Season	Autumn-winter n=33	Spring-summer n=67	0.76
Deficit	13 (39.3%)	31 (46.2%)	
Insufficiency	13 (39.3%)	25 (37.3%)	
Norm	7 (21.2%)	11 (16.41%)	

In the group of healthy people, in contrast to patients with CHC, there was a tendency to seasonality on the level of vitamin D. Thus, the analysis of vitamin D among healthy people (Table 3) showed that in most people (75% – in spring and summer and 50% – in autumn and winter) the optimal level of vitamin D is observed, however, in the spring-summer period during the highest solar radiation the normal level of vitamin D was registered 1.5 times more often.

Table 3

Analysis of vitamin D levels in the control group of healthy individuals depending on the season

Vitamin D status	Control group of healthy individuals n=30		p
Season	Autumn-winter n=14	Spring-summer n=16	
Deficit	2 (14.2%)	0	
Insufficiency	5 (35.7%)	4 (25%)	
∑	7 (49.9%)	4 (25%)	$p_{(a-w)-(s-s)}$ <0.05
Norm	7 (50%)	12 (75%)	$p_{(a-w)-(s-s)}$ <0.05
	P norm-disturbance >0.05	P norm-disturbance <0.05	

In autumn and winter, half of healthy people have a decrease in vitamin D content: a third of people (35.7%) – to suboptimal level (insufficiency), a small proportion of people (14.2%) – deficiency of vitamin D. In autumn and summer in healthy people vitamin D deficiency is not observed at all, and suboptimal levels are observed in a quarter of patients (25%). When calculating the odds ratio, no statistically significant dependence of vitamin D status on seasonality in healthy people was obtained (odds ratio =0.46, confidence interval 0.09-2.38). This fact indicates that healthy people do not have a serious risk of developing persistent disturbances of vitamin D metabolism; the decrease in vitamin D content in some healthy people is individual, and can quickly recover in the most favorable period of the greatest seasonal insolation (spring and summer).

Table 4

Comparative analysis of vitamin D levels in patients with CHC and in the control group of healthy individuals depending on the season

Vitamin D status	Groups				p
	patients with chronic viral hepatitis C n=100		control group n=30		
	1		2		
Season, n (%)	Autumn-winter n=33	Spring-summer n=67	Autumn-winter n=14	Spring-summer n=16	
Deficit	13 (39.3)	31 (46.2)	2 (14.2)	0 (0.0)	
Insufficiency	13 (39.3)	25 (37.3)	5 (35.7)	4 (25)	
∑	26 (78.6%)	56 (83.5%)	5(49.9)	4(25)	p1-2 autumn-winter <0.05 p1-2 spring-summer <0.05
Norm	7 (21.2)	11 (16.41)	7 (50)	12 (75)	p1-2autumn-winter >0.05 p1-2 spring-summer >0.05
	P norm-disturbance of vit.D<0.05	P norm-disturbance of vit. D<0.05	P norm-disturbance of vit. D>0.05	P norm-disturbance of vit. D<0.05	

A comparative analysis of the influence of seasonality on the content of vitamin D in both groups - patients with CHC and in the group of healthy individuals showed that subnormal levels of vitamin D are consistently registered in most patients with CHC (autumn-winter – 78.6%; spring-summer – 83.5%) regardless of the season, but in most healthy people the optimal level of vitamin D is observed (autumn-winter – 50%, spring-summer – 75%). The group of healthy people is characterized by a tendency of influence of the season on the content of vitamin D: in autumn and winter, half of people have a decrease in this indicator, often to suboptimal levels. The data are presented in the Table 4. The article investigated the influence of seasonal factor on the level of vitamin D in patients with chronic hepatitis C. The photobiological source of vitamin D in the human body is due to the synthesis of this vitamin in the skin mainly after insolation, in particular as a result of ultraviolet rays. In humans, this provides from 80% to 90% of the body's needs. V.V. Povoroznyuk and co-authors in their study "Level 25 (OH) of vitamin D in the adult population of different regions of Ukraine" showed that probably higher rates of 25 (OH) D in the southern regions of Ukraine were observed in the summer, especially in August. In our research studying the effect of seasonal factors on the level of 25 (OH) D in the serum of patients with chronic hepatitis C, we found that in patients with HCV, regardless of seasonality, a reduced level of vitamin D (p=0.31) was determined as compared with healthy (p=0.015), which may indicate a disturbance of vitamin D homeostasis in the liver (p<0.001). The calculation of the odds ratio also confirmed the lack of communication regardless of the season in patients with HCV (odds ratio =1.22, confidence interval 0.38-3.90). The most favorable time of the year, when the highest level of 25 (OH) D in serum was registered (Me (IQR) – 22.3 (17.5; 32.5) ng/ml), is also the spring-summer period. The maximum content of vitamin D in patients with CHC during this period was 62.61 ng/ml. The lowest level of 25 (OH) D in the serum was found in patients with chronic viral hepatitis C also in the spring-summer period (Me (IQR) – 22.3 (17.5; 32.5) ng/ml), Me corresponded to vitamin D deficiency. The minimum content of vitamin D in this period was 6.9 ng/ml. In the autumn-winter period, the level of vitamin D in patients was not significantly higher (p>0.05) than in the spring-summer period. Thus, patients with HCV, regardless of seasonality, had a reduced level of vitamin D (p=0.31) compared with healthy people (p=0.015), which may indicate a disturbance of homeostasis of vitamin D in the liver (p<0.001).

CONCLUSIONS

Analyzing the effect of endogenous route of vitamin D on the metabolism of cholecalciferol at different times of the year, we can conclude that the content of vitamin D in the serum of patients with chronic viral hepatitis C, regardless of the season, is lower than in healthy individuals, and the level of its decrease does not depend on seasonality (odds ratio =1.22, confidence interval 0.38-3.90). Subnormal levels of vitamin D are consistently registered in most patients in the autumn-winter period – 78.6% and spring-summer – 83.5%, exceeding the corresponding indicators of healthy individuals (p <0.001).

Conflict of interest. The authors declare no conflict of interest.

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