Role of Steatosis in the Pathogenesis and Course of Chronic Hepatitis C

Iu. Lupasco

Laboratory of Gastroenterology, Department of Internal Medicine
Nicolae Testemitanu State Medical and Pharmaceutical University, Chisinau

Abstract

Chronic hepatitis C – became a serious issue of public health due to its high prevalence throughout the world. According to the WHO data, in the next decades among the chronic diffuse diseases of the liver two basic pathologies that lead to the liver cirrhosis will predominate, - chronic hepatitis and steatosis. Patients with chronic hepatitis C infection have variable degree of steatosis even in the absence of other possible steatogenic factors, like alcohol, drugs or metabolic syndrome. According to studies of previous years it is believed that liver steatosis is more frequently encountered and has severe forms in chronic hepatitis caused by the genotype 3 of viral infection C, so-called “virus steatosis”, explaining this thereby that the replication of virus directly participates in lipid accumulation in the liver tissue. The direct responsibility of virus C in the pathogenesis of steatosis is shown by: 1. the association with chronic hepatitis C genotype 3 infection, suggesting that some viral sequences are involved in the intracellular accumulation of lipids; 2. the correlation between severity of steatosis and viral C replication levels; 3. association between response to treatment and disappearance of steatosis. Other observations suggest that the pathogenesis of steatosis in chronic hepatitis C is not caused only by virus C. The origin of the mild steatosis observed in most patients may be metabolic, since its severity correlates with body mass index and insulin resistance. Management of steatosis in chronic hepatitis C requires knowledge of its pathogenesis.

Key words: chronic hepatitis C, hepatitis C genotype, liver steatosis.

8. Кароли НА., Ребров АП. Хроническая обструктивная болезнь легких и инсулинемическая болезнь сердца. Клиническая медицина. 2005;6:72-76.

Corresponding author
Mahmoud Chikh Ahmad, Doctoral Student
Department of Internal Medicine
Nicolae Testemitanu State Medical and Pharmaceutical University
51, Puskin Street
Chisinau, MD-2012
Republic of Moldova
Telephone: 267024
Manuscript received December 15, 2010; revised manuscript January 31, 2011
Introduction

Chronic hepatitis C (CH-HCV) – became a serious issue of public health due to the high prevalence throughout the world (>200 mln. of the infected patients only regarding the anti-HCV analysis). According to the data presented by the WHO (World Health Organization), in the next decades among the chronic diffuse diseases of the liver (CDLD) two basic pathologies that lead to the liver cirrhosis (CP) will predominate - chronic hepatitis and steatosis [7].

Non-alcoholic fatty liver disease (NAFL) represents a spectrum of hepatic pathology ranging from simple steatosis to steatohepatitis (NASH) and to cirrhosis. NASH is a more advanced form of liver disease than NAFL, because in NASH steatosis is accompanied by hepatocyte injury and death, as well as hepatic infiltration of inflammatory cells. The frequency of steatosis according to the data of different studies in different groups of patients varies from 20-35% [4].

Hepatic steatosis, defined as excessive lipid accumulation in the cytoplasm of hepatocytes, is a frequent histological feature in patients with chronic hepatitis C (CHC) infection. Histological examination shows that these patients have variable degree of steatosis even in the absence of other possible steatogenic factors, like alcohol, drugs or metabolic syndrome [2]. In hepatitis C infected patients liver steatosis is mainly macrovesicular and is located in the periportal area rather than in the centrilobular area, in contrast to what is observed in non-alcoholic fatty liver disease and in alcoholic liver disease. Prevalence of liver steatosis in HCV patients is significantly higher when compared to patients with other forms of chronic liver disease such as hepatitis B or autoimmune hepatitis, suggesting a direct effect of HCV replication in the development of excess fat accumulation in the liver.

According to studies of previous years 1997-2004 it is believed that liver steatosis is more frequently encountered and has severe forms in chronic hepatitis caused by the genotype 3 of HCV infection, so-called “virus steatosis”, explaining this thereby that the replication of virus directly participates in the lipid accumulation in the liver tissue. Antiviral therapy in these patients contributed to the decrease of the liver steatosis. Meanwhile antiviral treatment in patients with different genotypes of HCV didn’t give the same results. Relapse in this case can be caused by the appearance of steatosis in patients with its absence or by the loading of the degree of steatosis in those, in whom it was minimal prior to the beginning of treatment. The mechanism of the triglycerides (TG) accumulation in liver can be caused: a) by damage of secretion, b) by the decrease of neo- synthesis, c) by the disturbance of degradation [6].

The progression NAFLD to NASH increases the risk for the development of cirrhosis and consequent liver-related morbidity and mortality. The rate of progression of chronic hepatitis C is variable, depending on many cofactors, mostly host-related, such as age, gender, alcohol consumption, overweight and co-infection. Steatosis has been recognized as one of these factors capable of influencing both liver fibrosis progression and the rate of response to interferon-alpha based therapy [6].

Literature data distinguish two main mechanisms of the formation of liver steatosis with different genotype of the virus of hepatitis C. In patients with the genotype 3 is present so-called “virus obesity” of the liver that develops also in absence of any other steatogenic factors and that seems to be directly triggered by the virus.

Liver steatosis is more frequent and more severe in HCV genotype 3, than in HCV genotype 1, presenting in 78% of patients infected with genotype 3 of HCV. The mechanism underlying this genotype specific lipid accumulation in the cytoplasm of HCV-3 infected hepatocytes is limited. This viral genotype more often and more significantly than other genotypes would be directly involved in the accumulation of triglycerides in hepatocytes. The first observation is about that, in patients with genotype 3 the severity of steatosis correlates with the level of HCV replication (expressed as HCV RNA level in liver (60) or in serum (1)). The second observation is extremely important and presents the fact of reducing or disappearing of liver fat accumulation in patients with HCV genotype 3 successfully treated with antiviral agents. The apolipoprotein B (apoB), triglyceride and cholesterol serum levels are significantly lower in patients with HCV-3 infection in comparison to patients chronically infected with other HCV genotypes, suggesting a profound alteration in lipid and lipoprotein metabolism in infected hepatocytes [8].

Chronic hepatitis C and lipid metabolism

It is generally believed that steatosis in the course of HCV viral inflammation can be caused by cellular metabolic disturbances that have been directly triggered by the virus [1 8]. The core protein or the HCV virus itself of both genotypes has been found in the hepatocytes, having a subcellular localization on the surface of lipid droplets mainly in macrovesicular pattern, which may confirm the cytopathic impact of the virus that leads to fact accumulation of hepatic cells. This viral protein localization on the surface of the lipid droplets and its over-expression seems to cause the further stimulation of lipid droplets formation in hepatocytes. These data suggest that interaction between HCV core protein and lipid droplets could contribute to steatosis.

In infected patients [1, 8], HCV particles circulate as low-density lipoprotein (LDL) - virus complexes rich in triglycerides. These so-called lipoviral particles (LVPs) were found to contain viral RNA, the viral structural proteins, core and envelope glycoproteins E1 and E2 (fig. 1). The reasons for the circulation of the virus with lipoproteins are not clear [5]. It is possible that this allows the virus to avoid the recognition by leukocytes and also provides a mechanism to enter cells as a surrogate along with lipoproteins. There is a strong evidence for an association between viral infection and lipoprotein metabolism.

According to the experimental model the HCV core protein seems to inhibit the microsomal triglyceride transfer protein (MTP) activity. Since this enzyme plays a key rate limiting role in very low-density lipid (VLDL) assembly, the consequence of its inhibition is the accumulation of triglycerides, and steatosis. A direct interaction between core
Fig. 1. Simplified schematisation of some of the factors participating in hepatitis C virus (HCV) lipoviroparticle (LVP) assembly.

protein and MTP is unlikely. However the MTP inhibition may still be indirect. Intrahepatic levels of MTP mRNA were reduced in patients with CHC, especially those with steatosis and genotype 3 [5].

Several mechanisms by which HCV and its proteins might cause excessive lipid accumulation has been suggested and widely discussed in many reviews. HCV core protein may accumulate in the mitochondria and induced damage via the production of reactive oxygen species (ROS). This can then block VLDL secretion. The intracellular accumulation of triglycerides may further contribute to the pathogenesis of steatosis by providing the fuel for continuing lipid peroxidation. HCV may also induce steatosis via ex novo synthesis of fatty acids. HCV may finally cause steatosis by impairing fatty acid oxidation. Chimpanzees infected with HCV show an increased intrahepatic activity of enzymes involved in lipogenesis, such as ATP citrate lyase which are regulated by SREBP-1c* (fig. 2).

Regulation of lipogenesis in hepatocytes (left) and adipocytes (right). The effects of nutrients and hormones on the expression of lipogenic genes are mostly mediated by SREBP-1 and, in adipose tissue, by PPARγ (peroxisome proliferator-activated receptor gamma). Lipogenesis entails a number of discrete steps, shown in the middle, which are controlled via allosteric interactions, by covalent modification and via changes in gene expression. (Sander Kersten, 2001)

Increased lipogenesis occurs via activation of specific transcription factors, largely documented in experimental models but so far not supported by the scanty findings in human livers. Impaired secretion of very low-density lipoprotein is supported by both experimental data and analysis of human liver tissue: in particular, microsomal triglyceride transfer protein (MTP) mRNA and activity seem reduced in case of steatosis. Decreased fatty acid (FA) oxidation is compatible with the reduced expression of peroxisome proliferator-activated receptor α (PPARα) in the liver of patients with chronic hepatitis C. Although an increased afflux of non-esterified fatty acids (NEFA) may lead to steatosis, there is no evidence that this occurs in patients with hepatitis C without the metabolic syndrome. The HCV core protein may additionally bind to and activate the retinoid receptor α (RxRα), α-transcriptional regulator that controls cellular lipid synthesis (10). The HCV core protein is followed by a reduced expression of peroxisome proliferator – activated receptor – α. (PPARα), a nuclear receptor regulating several genes responsible for fatty acid degrada-

Fig. 2. Mechanisms of nutritional and hormonal regulation of lipogenesis.

* SREBP-1c – signalling steroid-binding protein, transcription factor plays a central key role in the implementation of the insulin cascade. SREBP-1c protein also participates in the regulation of enzymes involved in lipogenesis “de novo”, takes part in the inhibition of beta-oxidation - two factors that lead to the accumulation of triglycerides in hepatocytes. At the same time stimulation of SREBP-1c-protein in vitro may lead to increased replication of HCV.
tion. These authors have also reported a down-regulation of mitochondrial carnitine palmitoyl transferase – 1 (CPT-1), the rate-limiting enzyme of mitochondrial β-oxidation, and of the acyl-CoA oxidase (AOX) in the liver of chronic hepatitis C patients (10). However, the down-regulation of CPT-1 and AOX is transcriptionally controlled by PPARα, which is significantly reduced in the liver of patients infected with genotype 3 HCV in comparison to genotype 1 (fig. 3).

The above mentioned data support the hypothesis that HCV core protein may modulate the expression of lipid degradation possibly via the down-regulation of PPARα. No single mutation has been identified as being responsible for steatosis suggesting that more complex mutation clusters may be involved in virally driven steatosis [1]. It is possible that the core protein of genotype 3a contains several unique mutations that alone, or more likely in combination, may confer the steatogenic phenotype [6].

HCV may interfere with lipid metabolism via at least three distinct, non-mutually exclusive mechanisms (fig. 4).

There is an opinion that in patients with the genotype of 1a is observed so-called “metabolic obesity” of the liver, and is associated with increased BMI, hyperlipidemia, and insulin resistance. In chronic hepatitis C patients who are infected with non-3a genotypes (genotype 1 infection) hepatic steatosis correlated with BMI (BMI higher than 25, but less than 36). This metabolic steatosis is not or very little modified by successful antiviral therapy. Visceral obesity rather, than merely increased BMI, seems to play a major role in the development of HCV-related steatosis. In patients with genotype non-3a (1) the most likely cause of steatosis is insulin resistance. Patients with a higher HOMA* score tend to respond less well to IFN-α, and a higher expression of the suppressor of cytokine signaling-3 (SOCS-3), an intracellular factor involved in both resistance to IFN-α and impaired insulin signaling, failing to respond to antiviral therapy.

(i) Impaired secretion. HCV may interfere with the very-low density lipoprotein (VLDL) assembly and/or secretion. Both apolipoprotein B (ApoB) secretion and microsomal triglyceride transfer protein (MTP) activity are impaired by HCV core protein expression.

(ii) Increased de novo synthesis of free fatty acids (FFA). HCV has been reported to upregulate sterol regulatory element binding protein (SREBP)-1c signalling pathway, leading to the up-regulation of enzymes involved in lipogenesis such as FA synthase (FAS).

(iii) Impaired FA degradation. The HCV core protein reduces the expression of peroxisome proliferators-activated receptor (PPAR)-α, a nuclear receptor regulating several genes responsible for FA degradation, as well as that of mitochondrial carnitine palmitoyltransferase type 1 (CPT)-1, the rate-limiting enzyme of mitochondrial β-oxidation. ACC: acetyl-CoA carboxylase; SCD: stearoyl coenzymeA desaturase. (Sophie Clement, 2009)

The mechanisms leading to steatosis in patients with chronic hepatitis C non-3a genotype are multiple. On one hand, free fatty acids overflow from adipose tissue to the liver is a direct consequence of the failure to block lipoprotein lipase, resulting in increased uptake by peripheral tissues, including liver. On the other hand, the deregulated hyperglycemic/hyperinsulinemic state stimulates the expression of a variety of enzymes involved in a fatty acids neosynthesis, and at the same time, inhibits the mitochondrial β-oxidation. Some degree of synergism may occur at the level of the above metabolic pathways. (CPT-1 inhibition). Insulin is known to be a down-regulator of ApoB synthesis. However recent data suggest that chronic insulin resistant state may stimulate MTP activity in order to increase hepatocyte VLDL output.

* The homeostatic model assessment (HOMA) is a method used to quantify insulin resistance and beta-ell function. It was first described under the name HOMA by Matthews et al. in 1985.
Presumably two mechanisms may operate in different groups of patients in hepatitis C. In those with genotype 3, who have the lowest levels of insulin resistance, hypotriglyceridemia, low ApoB serum levels and “viral”, steatosis as a final consequence. The second possible mechanism may occur in patients infected with genotype non-3 with hypertriglyceridemia, but the inability to counterbalance the stimulation of fatty acids neogenesis and the inhibition of mitochondrial β-oxidation will still result in “metabolic” steatosis. The level of insulin resistance may be genotype specific, since patients with genotype 3 had lower levels of HOMA scores than patients with genotype 1 [6].

HCV may induce liver steatosis by interfering with lipid metabolism in hepatocytes, and indirectly by influencing the level of insulin resistance. It has to be noted, however, that as many as 30% of patients with fatty liver who do not drink alcohol and are infected with genotypes other than 3a have normal BMI and HOMA score.

During the last few years a new interesting discussion appeared whether steatosis might be advantageous for the virus. Why HCV might want to induce steatosis? Is there a role for steatosis in HCV replication?

It is known that, in patients with “viral steatosis”, the severity of the fatty liver correlates with HCV replication level: in these patients, replication precedes fatty accumulation, and not vice-versa, as it was showed by antiviral treatment data. Conversely, in patients with “metabolic steatosis”, in whom steatosis precedes viral infection and proceeds independently of it, the level of viral replication is not increased in parallel with the severity of fatty liver [6].

**HCV, steatosis and fibrosis progression**

In recent years, scientists have debated the impact of steatosis on the occurrence of fibrosis in CHC (Tab. 1). Different opinions on interrelations between obesity, liver steatosis and fibrosis in patients with HCV are contradictory. Some investigators have reported that obesity induces progression of hepatic steatosis and fibrosis in HCV infected patients [2].

**Factors associated with rapid fibrosis progression in chronic hepatitis C (2)**

<table>
<thead>
<tr>
<th>Associated</th>
<th>Possibly associated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>Steatosis</td>
</tr>
<tr>
<td>Duration of infection</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>Long term excessive alcohol consumption</td>
<td>High HCV heterogeneity</td>
</tr>
<tr>
<td>Long term immunosupression (organ transplantation, bone marrow transplantation)</td>
<td>HCV genotype 3</td>
</tr>
<tr>
<td>HIV coinfection</td>
<td>Smoking (tobacco)</td>
</tr>
<tr>
<td>Overweight</td>
<td></td>
</tr>
<tr>
<td>High initial fibrosis stage</td>
<td></td>
</tr>
<tr>
<td>High initial necroinflammation grade</td>
<td></td>
</tr>
</tbody>
</table>

The epidemiologic studies suggest that the presence of diabetes mellitus and insulin resistance per se are risk factors of severe fibrosis in chronic hepatitis C. Steatosis grade appears to relate to hepatic fibrosis progression rate in chronic hepatitis C genotype non-3. Recent studies have suggested that the development of insulin resistance is affected by direct interference of HCV with the insulin cascade via proteasomal degradation of insulin receptor substrates-1-an-2.

This functional impairment of the insulin cascade is enhanced through increased levels of pro-inflammatory cytokines, including tumor necrosis factor (TNF) -α. TNF-α genotype modulates the activity of TNF-α pathway, influences insulin sensitivity and the severity of HCV chronic hepatitis (9). Euguchi G. et al (17) has proved that insulin resistance evaluated by HOMA-IR and QUICKI was correlated with visceral fat accumulation, and was higher in HCV patients than in NAFLD patients with visceral obesity. Serum-soluble TNF-receptors 1 and 2 were higher in HCV patients, than in NAFLD patients with visceral obesity. Author has concluded that the hepatitis C viral infection is a risk factor for development of insulin resistance particularly in patients with visceral obesity. According to Muzzi A. et al and others fibrosis is associated with insulin resistance in chronic hepatitis. Several studies have demonstrated that insulin resistance and visceral obesity impair the sustained response to peginterferon plus ribavirin in HCV-infected patients.

Adiponecint is a protein hormone with anti-inflammatory and anti-fibrosis effects, which could modulate glucose regulation and fatty acid catabolism. Thus, IR and adiponectin appeared to play an opposite role in this genotype-specific difference. In patients with chronic HCV a significant negative correlation was determined between serum adiponectin and male gender, body mass index and serum insulin. Adiponectin was associated with steatosis only in males and was paradoxically increased with inflammation. A positive correlation was found between serum adiponectin levels and viral loads in all HCV patients and IR.

There are different other factors besides IR that participate in fibrosis progression in patients with HCV with liver steatosis. In this regard, the literature discusses the leptin levels in chronic hepatitis C patients with CHC. In chronic hepatitis C the level of intrahepatic expression of CTGF (the connective tissue growth factor) was correlated with serum levels of leptin and the scores of steatosis and fibrosis. Since hepatic stellate cells possess leptin receptors exists a suggestion that leptin may represent the link between steatosis, insulin resistance and fibrosis in chronic hepatitis C.

Particular importance is attached to the processes of lipid peroxidation in patients with CHC and steatosis. The presence of hepatic steatosis, oxidative stress is enhanced in HCV infection and may promote fibrogenesis [10]. Kitase A. et al concluded that steatosis in CHC may amplify the oxidative stress-driven lipid peroxidation by providing the necessary "fuel" in the form of excess fat in the liver.

HCV can induce reactive oxygen species (ROS) via multiple mechanisms. The particular localization of the core protein within the outer membrane of mitochondria may induce increased oxidation of mitochondria glutathione (GSH) and facilitate the uptake of Ca2+ into the mitochondria by sensitizing mitochondria to mitochondrial permeability transition. There is an increase in ROS production by mitochondrial electron transport complex I (circles with roman letters, the
sites of ROS production in the mitochondrial electron transport chain have been localized in Complex I and Complex III and a redistribution of cytochrome C (cyt C) from the mitochondrial to cytosolic fractions. The HCV nonstructural proteins including NS5A are associated with the membrane of the endoplasmic reticulum (ER), which activates the release of Ca2+ from ER, thereby inducing oxidative stress. NS3 has been shown to trigger ROS production via activation of NADPH oxidase 2 (Nox2) (fig. 5).

Steatosis, apoptosis and HCV

A recent meta-analysis (the HCV MAI study), which included individual data of 3068 patients with chronic hepatitis C from 10 centers in five countries demonstrated that liver steatosis is associated with increased liver inflammatory activity. Pro-inflammatory cytokines activity may mediate fibrogenesis in the steatotic liver in patients with CHC. In chronic hepatitis C in the presence of steatosis, increased apoptosis, that is associated with necro-inflammatory activity, activation of stellate cells and increased stage of fibrosis. The caspase activity, which controls apoptosis is increased in both liver biopsy and serum from HCV patients and is strictly correlated to the extension of steatosis. All these data indicate a relationship between steatosis, apoptosis and development of fibrosis in CHC.

Does steatosis influences on the development of hepatocellular carcinoma? A recent study has reported steatosis to be an independent risk factor for the development of HCC (hepatocellular carcinoma) in CHC.

Steatosis has been recognized as a negative factor response to antiviral therapy in patients with non 3a genotype. Chronic hepatitis C patients who do not respond to interferon-alpha may have increased levels of suppressor of cytokine signaling 3 (SOCS-3) in the liver, a factor promoting the proteasomal degradation of IRS-1 and violate an adequate response to antiviral treatment in patients with CHC.

Conclusions

1. Steatosis is frequently associated with chronic hepatitis C, especially genotype 3, achieving 73% in this case.
2. In chronic hepatitis C two types of steatosis can be distinguished: “viral” in genotype 3 and “metabolic” in genotype non-3.
3. It has to be noted that the diagnosis of viral and/or metabolic steatosis in any patient should not be based only on the viral genotype, but should be elaborate using a whole set of clinical and laboratory parameters, including the assessment of the insulin resistance score.
4. In patients with chronic hepatitis C genotype 3 successful antiviral treatment is associated with disappearance or significant amelioration of liver steatosis.
5. In the occurrence of steatosis in HCV infection non-3 genotype LPO processes, an imbalance of adiponectin, pectin and signaling cytokines play a major role in addition to insulin resistance and visceral obesity.
6. The interrelation between chronic hepatitis C, steatosis with fibrosis and primary liver cancer was reported.

References

Corresponding author
Lupasco Iulianna, MD, Ph.D., Associate Professor Laboratory of Gastroenterology Department of Internal Medicine Nicolae Testemitanu State Medical and Pharmaceutical University 29, N. Testemitanu Street Chisinau, Republic of Moldova Telephone: 205539 E-mail: flowercat_2004@yahoo.com

Manuscript received November 25, 2010; manuscript revised January 26, 2011

Интенсивность перекисного окисления липидов и состояние антиоксидантной системы при вирусном гепатите В

М. А. Абдурахманова, А. М. Эфендиев

Кафедра биохимии, Азербайджанский медицинский университет, Баку

M. A. Abdurahmanova, A. M. Efendiev

*Intensivity of Lipid Peroxidation and Antioxidant Status in Hepatitis B*

We investigated 59 patients (41 men and 18 women). The control group included 20 healthy donors. The next biochemical indices were determined: common protein, alaninaminotrasferaz, spartatamintransferaz, alkaline phosphatase, lactatdehydrogenaz. It was determined that the most essential changes happened in the liver indices that lead to the cytolysis of hepatocytes. The intensity of lipid peroxidation by malonic aldehdyd, diene conjugates, superoxidismutaz, catalaz, ceruloplasmin were also studied. Results demonstrate that because of intensification of lipid peroxidation the antioxidant status of the body is changed.

**Key words:** hepatitis B viral, lipid peroxidation, antioxidants, superoxiddismutaz, catalaz, ceruloplasmin.

Реферат
Проведено исследование основных биохимических показателей при гепатите В. Была исследована кровь 59 больных (41 мужчина и 18 женщин). Контрольную группу составили 20 здоровых доноров. Из биохимических показателей были определены: общеполюбизм, аланинагидраз, аспартатагидраз, алкалиназа, лактатдеидрогеназа. Было выявлено, что значительные изменения происходят в печеночных показателях, что указывает на наличие цитолиза гепатоцитов. Также были изучены интенсивность перекисного окисления липидов (ПОЛ) и состояние антиоксидантной системы по таким показателям, как мальоновый диальдегид, диеновые конъюгаты, супероксиддисмутаза, каталаза, церулоплазмин. Полученные данные показали, что на фоне усиления ПОЛ происходит изменение антиоксидантного статуса организма.

**Ключевые слова:** вирусный гепатит В, перекисное окисление липидов, антиоксиданты, супероксиддисмутаза, каталаза, церулоплазмин.

Введение

Вирусные гепатиты относятся к наиболее социально-значимым проблемам медицины и всего человечества в целом, так как характеризуются непрерывным ростом заболеваемости и частым формированием неблагоприятных исходов (хронический гепатит, цирроз, карцинома и другие хронические процессы в организме) и смертностью, связанной как с острой, так и хроническими формами болезни [1, 2, 3].