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INSULIN RESISTANCE IN PATIENTS WITH CHRONIC HEPATITIS C

ИНСУЛИНСКА РЕЗИСТЕНЦИЈА КАЈ ПАЦИЕНТИ СО ХРОНИЧЕН ХЕПАТИТИС Ц

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Abstract

Introduction. Insulin resistance is the most common extrahepatic manifestation associated with hepatitis C virus, which leads to developing more pronounced fibrosis and liver steatosis. The aim of the study was to assess the prevalence of insulin resistance in non-diabetic, treatment naive patients with chronic hepatitis C and to analyze the relation of insulin resistance with genotype, viral load, gender, age, laboratory parameters, inflammatory and fibrotic changes in the liver, body mass index (BMI) and the presence of steatosis.
Methods. In this cross sectional study, 224 patients with hepatitis C viral infection were included. The patients were divided into two groups. The first group was with no insulin resistance and the second one with present insulin resistance. They were compared in terms of genotype, viral load, gender, age, inflammatory and fibrotic changes in the liver, BMI and liver steatosis.
Results. Insulin resistance was present in 45.5% of patients. The following factors were associated with insulin resistance: age (p=0.0022), inflammatory and fibrotic changes in the liver (p=0.001, p=0.006, respectively), steatosis (p=0.015) and transaminase activities (for AST, p=0,002, for ALT, p=0.001).
Conclusion. In the Republic of Macedonia, a high percent of 45.5% among non-diabetic and treatment naïve patients with chronic viral hepatitis C, had insulin resistance. Insulin resistance was more prevalent in older patients, in those with more pronounced inflammatory and fibrotic changes in the liver, in patients with steatosis and in those with higher transaminase activity.

Keywords: chronic hepatitis C, insulin resistance, steatosis, inflammation, fibrosis, body mass index

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Апстракт

Вовед. Инсулинската резистенција претставува најчеста екстрахепатична манифестација, асоцирана со вирусот хепатитис Ц, која е асоцирана и со развој на поизразена фиброза и стеатоза на црниот дроб. Цел на студијата е процена на застапеноста на инсулинската резистенција кај пациенти со хроничен хепатитис Ц, кои не се дијабетичари и кои досега не се лекувани, како и анализа на асоцираноста на инсулинската резистенција со генотипот, виремијата, полот, возраста, лабораториските параметри, инфламаторните и фибротични промени на црниот дроб, индекс на телесна маса и присуството на стеатоза.
Методи. во оваа студија на пресек се вклучени 224 пациенти со вирусна инфекција хепатитис Ц. Пациентите се поделени во група без инсулинска резистенција и во група со присутна инсулинска резистенција, кои потоа се споредувани во однос генотип, виремија, пол, возраст, инфламаторни и фиброзни промени во црниот дроб, индекс на телесна маса и стеатоза на црн дроб.
Резултати. Инсулинска резистенција е присутна кај 45.5% од пациентите. Фактори кои се асоцирани со инсулинска резистенција се возраста (p=0.0022), воспалителните и фиброзни промени во црниот дроб (p=0.001, p=0.006, соодветно), стеатозата (p=0.015) и трансаминазната активност (за АСТ p=0,002, за АЛТ p=0.001).
Заклучок. Постои висок процент на присуство на инсулинската резистенција од 45.5% меѓу пациентите со хроничен вирусен хепатитис Ц во Република Македонија, кои не се дијабетичари и кои досега не се лекувани. Инсулинската резистенција е позастапена кај постари пациенти, кај оние со поизразени инфламаторни и фибротични промени во црниот дроб, како и кај пациентите со присутна стеатоза и со зголемена трансаминазна активност.

Клучни зборови: хроничен хепатитис Ц, инсулинска резистенција, стеатоза, инфламација, фиброза, индекс на телесна маса

Introduction

Chronic hepatitis C virus infection is widespread throughout the world, affecting approximately 2.8% of the population, or about 185 million people worldwide are infected with the disease [1].
Impaired glucose metabolism is often seen in patients with chronic hepatitis C (CHC), so diabetes mellitus type 2 (DM) is the most common extrahepatic manifestation associated with hepatitis C virus (HCV) [2-3]. This HCV-diabetes association is due to insulin resistance (IR). Basically, IR is a pathological condition in which cells, especially those of adipose tissue, the muscles and the liver do not respond appropriately to insulin secreted by the pancreas. Thus, glucose cannot be absorbed from the circulation, which increases its level in the blood and stimulates the pancreas to secrete larger amounts of insulin in order to reduce serum glucose. Insulin resistance, pre-diabetes and finally, type 2 diabetes mellitus (DM) as known pro-atherogenic conditions are risk factors for developing atherosclerosis and cardiovascular events in this group of patients [4]. Unlike chronic hepatitis B, impaired glucose metabolism is often found in patients with hepatitis C virus infection. IR is also associated with the development of pronounced fibrosis and liver steatosis [5-9]. Mechanisms for HCV induced IR are several, such as the role of tumor necrosis factor-alpha (TNF-α) and the direct effects of HCV core protein in inhibiting insulin signaling pathway [10-13]. The presence of IR leads to a lower rate of sustained virological response (SVR) [14]. The reason for this negative association is not completely known, but some possible mechanisms have been mentioned: HCV core protein by stimulating the suppressor of cytokine signaling-3 (SOCS-3), which is a negative regulator of interferon-α (IFN-α) signaling; obesity by modulating INF signaling pathway, as well as increasing the lipid droplets in hepatocytes or resulting in poor lymphatic circulation [15]. Achieved SVR has a positive impact on the IR reduction [14]. Long duration of infection associated with metabolic abnormalities is the main reason for development of more advanced forms of liver damage. All these result in cirrhosis, requiring liver transplantation [16]. Hepatocellular carcinoma (HCC) is often associated with this type of infection, but also IR is associated with the development of HCC in patients with chronic HCV infection [17-18].
The primary goal of the study was to assess the prevalence of insulin resistance expressed by fasting plasma glucose (FPG), fasting level of the insulin in the blood and Homeostasis Model Assessment of Insulin Resistance (HOMA IR) in non-diabetic, treatment naive patients with chronic hepatitis C. Secondary endpoints analysis was the association of IR with genotype, viremia, gender, age, laboratory parameters (transaminases, lipid and carbohydrate status, C-reactive protein-CRP, ferritin and serum iron), histological changes in the liver (inflammatory and fibrotic), body mass index and the presence of steatosis.

Material and methods

In this observational and cross-sectional study, with prospective inclusion of data, a total of 224 non-diabetic patients with chronic hepatitis C were included, in the period from January 2010 to December 2015. The study was approved by the local Ethics Committee.
Inclusion criteria: treatment naïve, hepatitis C virus ribonucleic acid (HCV RNA) positivity patients, confirmed by PCR method.
Patients were excluded from the study if they were: co-infected with other virus (hepatitis B virus-HBV or human immunodeficiency virus-HIV), if they had other liver disease (autoimmune hepatitis, Wilson's disease, hemochromatosis, primary biliary cirrhosis, primary sclerosing cholangitis), signs of decompensation, history of liver transplantation, end-stage renal disease, type 2 diabetes mellitus, alcohol abuse (>20 g/day) and hepatocellular carcinoma.
Blood was taken from all patients and the samples were sent to central biochemical laboratory for analysis of the following parameters: transaminase activity (aspartate transaminase-AST, alanine aminotransferase-ALT), lipid status (triglyceride-TG, total cholesterol, high-density lipoprotein cholesterol-HDL-C; low-density lipoprotein cholesterol-LDL-C), FPG and fasting insulin blood level, hemoglobin A1c-HbA1c, CRP, ferritin and serum iron. Insulin resistance was calculated according to the formula of HOMA-IR: fasting insulin (μU/mL) x fasting glucose in plasma (mmol/L) /22.5.). For the value of ≥2 insulin resistance was confirmed.
The genotyping and the viremia were performed at the Research Centre for Genetic Engineering and Biotechnology "Georgi D. Efremov", Macedonian Academy of Sciences and Arts.
Assessment of inflammatory activity and liver fibrosis was made by a liver biopsy. Knodell scale was used for measuring the degree of inflammation (HAI-histological activity index, which is numbered 1 to 18) and presence of fibrosis). Patients were divided into three groups: group 1-no fibrosis, group 2-evident fibrosis and 3-liver cirrhosis. Ultrasound was used to assess the presence of fatty liver. Patients were divided into three groups: group 0-no steatosis, group 1-mild steatosis and group 2-severe steatosis.
Patient weight was expressed through body mass index-BMI, which was calculated by the formula: weight in kg/height^2 in meters.
Patients in this study were divided into two groups: group 1-patients with hepatitis C virus infection with no evidence of IR and group 2-patients with hepatitis C virus infection with evidenced IR.
These two groups were compared in terms of multiple parameters such as gender, age, genotype, viral load, inflammatory and fibrotic changes in the liver, presen-

ce of steatosis, BMI and laboratory parameters (AST, ALT, TG, total cholesterol, HDL-C, LDL-C, fasting glucose and fasting insulin level in blood, HbA1c, CRP, ferritin and serum iron).

Statistical analysis: The statistical program SPSS 17 for Windows was used. For description of quantitative variables descriptive statistics was used (mean, standard deviation, standard error, median and interquartile range). For description of categorical variables, frequencies and percentages were used. For testing the difference of categorical variables between the two groups, Chi-square test was used. For testing the difference of numerical variables, Mann-Whitney test was used. For all analyzes p value <0.05 was considered statistically significant, and p <0.01 highly significant.

Results

Basic features of patients with chronic hepatitis C are shown in Table 1. Their average age was 33.83±8.24, and the values for FPG, fasting insulin level in blood, HOMA IR and HbA1c were: 5.24±0.74, 12.89±15.60, 2.87±3.44 and 5.22±0,94, respectively. Average value of BMI was 24.47±4.32. Insulin resistance was evidenced in many of the patients with chronic hepatitis C, in 102 patients (45.5%) of a total of 224 patients. The remaining 122 patients or 54.5% were patients with no evidence of IR (Table 2). With regard to gender males predominated in both groups of patients. Still, IR was more often evidenced in females (50.88%), compared to males (43.71%), with no statistical significance between the groups (p=0.348). The age of patients with IR was significantly higher (35.9±9.3) compared to patients with no evidence of IR (32.1±6.7), with significant difference of p=0.0022. In terms of genotype and viral load, no significant difference between the groups was detected (p=0.742 and p=0.900, respectively). Highly significant difference between the groups was obtained regarding inflammatory activity obtained from liver biopsy. Evidently, patients with determined IR had a higher Knodell score (HAI), with a mean HAI of 4.072±2.920, compared to those without IR whose mean HAI was 2.761±2.402 (p=0.001). Patients with evidenced fibrosis or cirrhosis more frequently encountered IR (in 54.05% and 83.33%, respectively), with statistical significance

Table 1. Baseline Characteristics of Patients With Chronic Hepatitis C Infection

Variable	Patients N=224
Age, years, mean ± SD	33.83±8.24
FPG (3.6-5.6 mmol/L), mean±SD	5.24±0.74
Fasting insulin (2-17 μIU/ml), mean±SD	12.89±15.60
HOMA IR, mean±SD	2.87±3.44
HbA1c (4.8-5.9%), mean±SD	5.22±0,94
BMI, mean±SD	24.47±4.32

Abbreviations: SD: standard deviation; FPG: Fasting plasma glucose; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, BMI: body mass index

Table 2. Absence or evidence of insulin resistance (IR) in patients with HCV

Baseline Characteristics	Absence of IR (N=122)	Evidence of IR (N=102)	P value
Sex, No (%)			
Male	94(56.29)	73(43.71)	0.3483 NS[1]
female	28(49.12)	29(50.88)	
Age, years, mean ± SD	32.1±6.7	35.9 ±9.3	0.0022 S[2]
Genotype No (%)			
Subtype 1	37(56.92)	28(43.08)	
Subtype 2	1(33.33)	2(66.67)	0,742 NS[1]
Subtype 3	80(55.56)	64(44.44)	
Subtype 4	1(33.33)	2(66.67)	
HCV viral load (IU/ml), mean±SD	2410226±6702822	2065677±5290369	0,900 NS[2]
Liver biopsy			
Knodell Histology			
Activity Index-HAI, mean±SD	2,761±2,402	4,072±2,920	0,001 S[2]
Presence of fibrosis, No (%):			
No fibrosis	100(60.24)	66(39.76)	
Fibrosis present	17(45.95)	20(54.05)	0,006 S[1]
Cirrhosis	2(16.67)	10(83.33)	
Steatosis, No (%):			
No steatosis	53(59.55)	36(40.45)	
Mild	29(40.85)	42(59.15)	0,015 S[1]
Severe	2(22.22)	7(77.78)	
BMI, mean ± SD	23.9 ±3.9	25.2 ±4.7	0.057 NS[2]

Legend: IR: insulin resistance; HCV: hepatitis C virus; SD: standard deviation; NS: not statistically significant; S: statistically significant; BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate transaminase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.
[1] Pearson Chi-square; [2] Mann-Whitney U Test

Table 3. Absence or evidence of insulin resistance (IR) in patients with HCV

Biochemical Characteristics	Absence of IR (N=122)	Evidence of IR (N=102)	P value
AST (10-34 U/L), mean±SD	56.9±46.2	74.9±71.4	0.002 S[1]
ALT (10-45 U/L), mean±SD	87.9±84.1	113.7±87.9	0.001 S[1]
Triglyceride (0.0-2.0 mmol/L), mean ± SD	1.1±0.6	1.3±0.8	0.142 NS[1]
Cholesterol (0.0-5.5 mmol/L), mean ± SD	4.2±1.1	4.2±1.2	0.732 NS[1]
HDL (0.9-2.0 mmol/L), mean±SD	1.2±0.3	1.1±0.3	0.157 NS[1]
LDL (2.2-3.7 mmol/L), mean±SD	2.5±0.9	2.5±1.01	0.801 NS[1]
FPG (3.6-5.6 mmol/L), mean±SD	5.0±0.6	5.5±0.8	0.0001 S[1]
Fasting insulin (2-17 μIU/ml), mean±SD	5.04±2.4	22.3±19.2	0.0001 S[1]
HOMA score, mean±SD	1.02±0.5	5.1±4.1	0.0001 S[1]
HbA1c (4.8-5.9%)	4.9±0.6	7.3±13.1	0.0001 S[1]
CRP	3.1±6.3	2.4±4.2	0.418 NS[1]
ferritin (up to 300 μg/L)	136.9±134.6	165.1±149.1	0.159 NS[1]
iron (7-28 μmol/L)	18.7±8.4	21.6±9.2	0.110 NS[1]

Abbreviations: IR: insulin resistance; HCV: hepatitis C virus; SD: standard deviation; S: statistically significant; NS: not statistically significant; ALT: alanine aminotransferase; AST: aspartate transaminase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FPG: Fasting plasma glucose; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, CRP: C-reactive protein; HbA1c: Hemoglobin A1c. [1]Mann-Whitney U Test

of p=0.006. Also, less severe as well as severe steatosis was more frequently expressed in patients with IR, with significant difference of p=0.015. It is clear that the IR group had higher body weight or higher BMI (25.2±4.7), unlike the other group (23.9±3.9), but there was no statistical difference (p=0.057) between the two groups. Mean value of AST and ALT in the group with IR was 74.9±71.4 U/L and 113.7±87.9 U/L, respectively, while in the group with no IR 56.9±46.2 U/L and 87.9±84.2 U/L, respectively. Highly significant difference between the groups (p=0.002 and p=0.001, respectively) was detected with regard to absence or evidence of insulin resistance. (Table 3).

There was no significant difference between the two groups in terms of triglycerides, total cholesterol and its fractions HDL-C and LDL-C. Fasting glucose level and fasting insulin level were significantly higher in the group with IR, as well as HbA1c. Thus, p=0.0001 refers to all three parameters. In a large number of patients FPG was between 5.6 and 6.9 mmol/L (in 29.9%) and also HbA1c was higher than 5.7% in 17.1% of patients, which means those patients can be included in the group of pre-diabetes according to the criteria of the American Diabetes Association. Analysis of CRP, serum ferritin and serum iron, as markers of inflammation, showed no significant statistical difference between the groups with or without IR (p=0.418, p=0.159 and p=0.110, respectively).

Discussion

The aim of our study was to show the presence of insulin resistance in non-diabetic, treatment naive patients with chronic hepatitis C in the Republic of Macedonia as well its relation with other factors that can further affect disease progression to fibrosis and cirrhosis.

We can clearly see the high prevalence of IR in up to 45.5% of patients. High representation of IR can also be found in other studies, such as the study of Kiran *et al.* (2013), the study of Moucari *et al*. (2008) where IR was found in 35% of patients with CHC, unlike the group with chronic hepatitis B (CHB) where IR was found in 5% of patients [19-20]

The important factors in relation to IR were as follows: age, extent of inflammatory and fibrotic changes in the liver, steatosis, transaminase activity, fasting glucose, insulin level and HbA1c value.

The age of the patients in our study was significantly higher in the group with IR (35.9±9.3), compared to the other group (32.1±6.7), with a significant difference of p=0.0022. This may be due to persistence of viral infection (longer influence of the virus) that contributed to the development of glucose metabolism disorder on one hand, but also evidence of metabolic disorders in the aged population of patients, on the other hand.

In our group of patients genotype 1 and 3 predominated, while the other two genotypes, 2 and 4, were found in a small number, which was not adequate for statistical analysis. Evidence of IR was almost identical in the two most common genotypes 1 and 3 (in patients with genotype 1 IR was found in approximately 43.08% and in patients with genotype 3 in approximately 44.44% of patients). Opposite to our results, there are studies indicating that genotype 1 and 4 were more often associated with IR [20].

In our analyzed group, there were no significant differences between the two groups in terms of viral load, which would mean that the number of virus particles was not associated with the IR, as also shown in the study of Huang *et al*. (2011) [21]. Unlike our study, in the study of Hsu *et al*. (2008) association between viremia and IR was shown [22].

Hepatitis C viral infection leads to the activation and the presence of inflammatory cells in the liver, which are responsible for the progress of the inflammatory condition which in turn causes liver damage and development of fibrosis. In our study more pronounced inflammatory and fibrotic changes in the histological preparation of the liver biopsy were found in IR group, with statistical significance of p=0.001 and p=0.006, respectively. In the study of Hickman *et al.* (2003), insulin was independently associated with fibrosis, but not with the inflammation [23].

There was a statistical significance in relation to the evidenced steatosis in patients with IR (p=0.015). Steatosis is an important co-factor which could result in accelerating the development of hepatic fibrosis and increased necro-inflammatory activity [24]. Steatosis in patients with genotype 3 is considered to be of viral origin ("viral steatosis") and is closely associated with viremia, while in other genotypes, it is associated with the factors of the host (obesity (particulary visceral), IR and type 2 diabetes mellitus ("metabolic steatosis") [25].

In our study patients deployed to the group of IR had greater weight, actually higher BMI (25.2±4.7) compared to the other group (23.9±3.9), but there were no statistical differences (p = 0.057) between these two groups. In the study of Souza *et al*. (2011) BMI was noted as a factor that was associated with IR, along with age and waist circumference [26]. Increased body weight, especially visceral adiposity along with other metabolic factors such as IR are the factors that lead to a greater degree of inflammatory activity (adipose tissue actually represents an organ where proinflammatory cytokines are excreted), pronounced steatosis and risk of disease progression to fibrosis [24,26].

The transaminases values (AST, ALT) were significantly higher in patients with IR, as opposed to those without IR, for p=0.002 and p=0.001, respectively. In general, increased transaminases value means a greater degree of hepatocellular damage that can be considered as an inviolable part of steatosis. Since our study showed a greater prevalence of steatosis in IR group (p=0.015), it cannot be strictly determined whether the increased transaminase activity in the group of IR patients was due to steatosis or the insulin resistance itself.

Regarding lipids, there was no significant difference between groups (with or without IR), while glucose status, fasting glucose level and fasting insulin level were significantly higher in the group with IR, as well as HbA1c, for p=0.0001 for all three parameters. In a rather large number of patients (29.9%) the value of FPG was between 5.6 and 6.9 mmol/L and also the value of HbA1c was greater than 5.7% in 17.1% of the patients. According to the criteria of the American Diabetes Association these patients enter the group of pre-diabetes, with real risk of developing type 2 diabetes mellitus.

In the situations of developed insulin resistance, increased production of inflammatory cytokines occurs, which improves the inflammatory damage of the hepatocyte. Serum ferritin and the CRP are markers of inflammation that are increased in terms of the inflammatory condition. In our study the values of ferritin and serum iron were higher in the group of IR, but there was no significant difference between the two groups in terms of CRP, ferritin and serum iron (p=0.418, p=0.159 and p=0.110, respectively) [27].

Conclusion

There was a high percentage of evidenced IR by 45.5% in CHC patients who were treatment naive and non-diabetic in R. Macedonia. IR was associated with the age, the inflammatory and fibrotic changes in the liver, with steatosis and transaminase activity or with other words, IR was more prevalent in older patients, those with a more pronounced inflammatory and fibrotic changes of the liver, patients with steatosis and higher transaminase activity. In the future, it has to be proved whether changes in metabolic factors which include IR will influence on the reduction of inflammatory and fibrotic activity and will prevent disease progression.

Conflict of interest statement. None declared.

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