ARTICLE ORIGINAL

The risk of subclinical carotidatherosclerosis in patients with chronic hepatitis C

Le risque d'athérosclérose carotidienne infra clinique chez les patients atteints d'hépatite C chronique

Mouna Medhioub[1], Khaled Bouzaidi[2], Asma Bachali[3], Amal Khsiba[1], Lamine Hamzaoui[1], Mohamed Moussadek Azouz[1]

1-Gastroenterology department
2-Radiology department
3-Biochemistry laboratory
Mohamed Taher Maamouri Hospital, Nabeul -Tunisia. / Université de Tunis el Manar, faculté de médecine de Tunis,

RÉSUMÉ

Introduction: Plusieurs études ont suggéré le rôle du virus de l'hépatite C dans la l'étiopathogénie de la maladie athéroscléreuse.
Objectif: Evaluer l'association entre l'athérosclérose carotidienne infra clinique et l'hépatite chronique C.
Méthodes: On a effectué des mesures anthropométriques et des bilans métaboliques pour 40 patients atteints d'hépatite C chronique et 40 cas contrôles . Le risque d'athérosclérose infraclinique a été évalué par la mesure échographique de l'épaisseur de l'intima-média carotidienne. L'athérosclérose à haut risque cardiovasculaire était définie par une épaisseur de l'intima-média carotidienne supérieure à 75% percentile.
Résultats: L'épaisseur de l'intima-média carotidienne et la prévalence de l'athérosclérose à haut risque cardiovasculaire étaient significativement plus élevées dans le groupe des patients par rapport au groupe contrôle (0,68 VS 0,60, p=0,02) et (82,5% vs 40%; 0,001) respectivement. L'activité ≥ A2 et l'âge> 40 ans étaient les facteurs indépendants associés à l'épaisseur de l'intima-média carotidienne et l'hépatite C était le seul facteur indépendant associé à l'athérosclérose à haut risque cardiovasculaire (OR = 4,81 à 95%: 1,6 à 14,42) .
Conclusion: Dans notre étude, l'hépatite C chronique était identifiée comme facteur de risque de l'athérosclérose infraclinique à haut risque cardiovasculaire.
Mots clés : Hépatite chronique C, athérosclérose, intima-média carotidienne, Insulino-résistance, risque cardio-vasculaire.

SUMMARY

Background : the role of hepatitis C virus in the pathogenesis of atherosclerotic disease has been suggested by several studies. We aimed to assess the association between subclinical carotid atherosclerosis and chronic hepatitis C .
Methods : 40 patients infected with chronic hepatitis C and 40 contol cases were evaluated by anthropometric and metabolic measurements .The risk of subclinical atherosclerosis was assessed by ultrasound measurement of carotid intima-media thickness . A high cardiovascular risk atherosclerosis was defined by carotid intima-media thickness > 75th percentile.
Results: The carotid intima-media thickness and the prevalence of high cardiovascular risk atherosclerosis were significantly higher in the group infected with hepatitis C compared to the control group (0.68 VS 0.60, p = 0.02) and (82.5% vs. 40%; 0.001) respectively. In multivariate studies, activity ≥ A2 and age> 40 years were the independent factors associated with the carotid intima-media thickness and hepatitis C was the only independent factor associated with high cardiovascular risk atherosclerosis (OR = 4.81 CI at 95%: 1.6-14.42).
Conclusions : In our study , chronic hepatitic C was associated with a high risk of carotid atherosclerosis .
Key- words : Chronic hepatitis C, atherosclerosis, carotid intima-media, Insulin resistance , cardiovascular risk .

Correspondance
Mouna Medhioub
Gastroenterology department, Mohamed Taher Maamouri Hospital, Nabeul -Tunisia. /Université de Tunis el Manar, faculté de médecine de Tunis
medhioub.mouna@yahoo.fr

INTRODUCTION

The prevalence of hepatitis C virus (HCV) worldwide is estimated at 1% (1). HCV is thought to be involved in insulin resistance (IR), diabetes and in atherosclerotic disease(2,3).

HCV is associated with several atherogenic conditions such as IR (4), steatosis (5) and chronic inflammation (3). Some studies have also shown that HCV replicates in carotid plaques causing vascular inflammation (6,7).

Measurement of carotid intima-media thickness (CIMT) with B-mode ultrasound is a sensitive technique for identifying atherosclerotic burden and cardiovascular disease (CVD) risk (8,9).

In our study, we evaluated the risk of carotid subclinical atherosclerosis in patients with chronic hepatitis C, compared with a control group.

METHODS

Our study was conducted from March 2015 to June 2017. In all, 40 patients with chronic hepatitis C (CHC) were prospectively recruited at the hepato-gastro-enterology department of Mohamed Taher Maamouri hospital. The inclusion criteria were: the presence of anti-HCV and HCV RNA without any features of acute hepatitis C. All patients met the following exclusion criteria: 1) Age ≤ 18 years, 2) cirrhosis Child Pugh B or C , 3) Hepatocellular carcinoma, 4) associated chronic liver disease: alcoholic hepatitis, hepatitis B, auto -immune hepatitis, primary biliary cholangitis , Wilson's disease, hemochromatosis, alpha antitrypsin deficiency, 5) alcohol consumption> 30g / day in men and> 20g / day in women, 6) co-infection with human immunodeficiency virus, 7) the use of steatosis-inducing drugs (corticosteroid, tamoxifen, amiodarone) or drugs interfering with lipid metabolism (statin, fibrate), 8) previous hepatitis C anti-viral treatment , 9) previous history of symptomatic atherosclerotic disease, 10) illicit drug addiction.

Forty patients, matched for sex, age, and body mass index (BMI) with the CHC group, were enrolled as controls. They were recruited at our department and suffered from gastroesophageal reflux disease and irritable bowel syndrome**.** All of them had negative anti-HCV and met the exclusion criteria noted above.

Clinical and biological data:

Clinical, anthropological and biological data were collected at the time of inclusion.

The BMI was calculated according to the following formula: BMI = weight (kg) / height (m^2). Patients were classified as obese if BMI> 30 kg / m^2.

Biochemical analyses were performed in the same central laboratory for all patients, including cholesterol (CT), HDL-cholesterol (HDL-CT), triglycerides (TG), glycemia and insulinemia.

Insulin resistance (IR) was evaluated by the index of homeostasis model assessment of insulin resistance (HOMA-IR) (10).

The criteria of the International Diabetes Federation (11) were adopted for the definition of the metabolic syndrome (MS).

Virological data:

The viral load of HCV (HCV –RNA) was measured by quantitative PCR in real time and expressed in IU/ml. The viral genotype C was determined by the viral sequencing method.

Evaluation of chronic hepatitis C fibrosis *:*

The inflammatory necrotic activity of hepatitis C was determined with fibrotest actitest test (12). It was performed only in patients with no clinical and biological signs of cirrhosis. Fibrosis was considered significant if ≥ F2.

Evaluation of the risk of atherosclerosis:

The CIMT was evaluated by an expert radiologist, using a high-resolution B-mode ultrasonography equipped with a multifrequency linear probe (8,9). CIMT was measured manually, at the left and right distal common carotid artery at 1 cm from the carotid bifurcation, on a segment measuring at least 10 mm. The CIMT maximum ($CIMT^{max}$) was defined as the highest CIMT measurements from the left and right side.

The diagnosis of Infra-clinical atherosclerosis with a high cardiovascular risk was based on CIMT max> 75th percentile ($CIMT^{75}$)(8,13).

Statistical study

The statistical analysis and data processing were performed with SPSS software (SPSS for Windows, version 22).

The Continuous variables, expressed on average ± standard deviation, were compared with Student t-test. The categorical variables, expressed on frequency, were compared with the chi 2 test and the exact test of Ficher.

To investigate factors associated with infra-clinical atherosclerosis, a univariate study and a multivariate study were conducted with the dependent variables $CIMT^{max}$ and $CIMT^{75}$.

For the univariate study, we used the ANOVA test for qualitative variables and bivariate correlation for quantitative variables. Differences were considered statistically significant if $p<0.05$. The correlation was classified as low (Bravais-Pearson r coefficient is close to 0), strong (r approaches +1 or -1) and no correlation (r = 0).

Variables associated with the dependent variable at univariate analysis (P < 0.10) were included in the multivariate regression models: linear regression model for the dependent variable $CIMT^{max}$ and the binary logistic regression model for the dependent variable $CIMT^{75}$ were used. The associations were expressed as odds ratios (OR) with their confidence intervals (95% CI).

The study was performed in accordance with the principles of the Declaration of Helsinki and its appendices and with local and national laws.

RESULTS

The distribution of baseline characteristics of the two groups were detailed in Table 1. The mean age of the CHC patients was 55±15.9 years. Twenty-six CHC patients were over 50 years. The two groups were comparable for age, sex, BMI, co-morbidities, smoking, and anthropological data.

Insulinemia and HOMA-IR index were significantly higher in the CHC group (p=0,005 and p=0,001 respectively). The CT and HDL-CT were significantly lower in the CHC group (p = <0.001 and p = 0.012 respectively). Fifteen patients and 6 controls had a MS (37.5% vs. 15%, p=0.02).

The predominant genotype OF HCV was 1 (92.5%). Significant fibrosis was noted in 27 patients (67.5%) and 15 patients (37.5%) had severe fibrosis (F3/4).

The mean value of $CIMT^{max}$ was significantly higher in the CHC group compared to the control group (0.68 VS 0.60 mm, p = 0.02).

The prevalence of $CIMT^{75}$ was significantly higher in the CHC group compared to the control group (82.5% vs 40%, p <0.001).

Factors associated with the $CIMT^{max}$

$CIMT^{max}$ was weakly correlated with age (r=0.48, p<0.001) but significantly increased in age ≥ 40 years (0.67 VS 0.47mm, p<0.001). HCV infection was associated with higher values of the $CIMT^{max}$ (Table 1). Significant fibrosis was associated with a higher $CIMT^{max}$in the CHC group. The value of the $CIMT^{max}$ was comparable between the control and CHC group with non-significant fibrosis (fig n°1). For necrotico-inflammatory activity, stage ≥ A2 was associated with a higher $CIMT^{max}$ (0.76 VS 0.61 mm, p = 0.004).

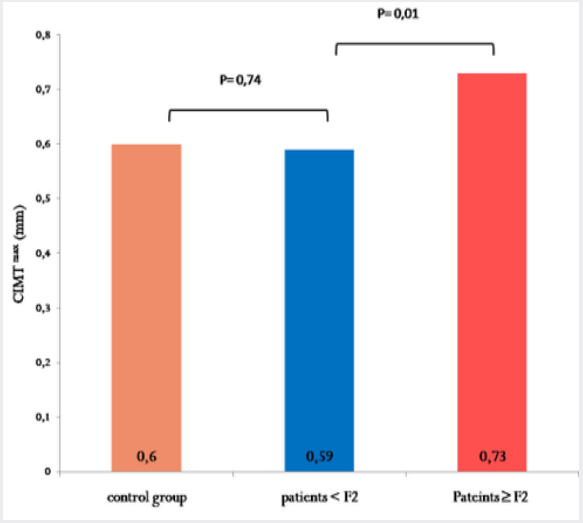

Figure 1. The CIMT max in CHC group and control group Patients with fibrosis < F2 had a mean CIMT max value comparable to the control group, but significantly lower than patients with fibrosis ≥ F2

The HOMA -IR index was weakly correlated with the $CIMT^{max}$ (r=0.31, p=0.005). On the other hand, there was no association between $CIMT^{max}$, the SM (p=0,17) and IR (p=0.5).

Table 1. Baseline demographic, laboratory,and metabolic features in heptitis C group and control group

Variables	CHC* group (n=40)	Control group (n=40)	p
Mean age, (years)	55 ±15,9	51,9 ±12,46	0,26
Gender (H/F)	13/27	17/23	0,36
diabetes (N/%)	7(17,5%)	3(7,5%)	0,18
Arterial hypertension (N/%)	10 (25 %)	15(37,5%)	0,22
dyslipidemia (N/%)	0	1(2,5%)	-
Smoking (N/%)	9(22,5%	6(15%)	0,57
BMI * (Kg/m²)	27,26 ±5,15	26,37±4,51	0,42
Obesity	12(30%)	9(22,5%)	0,55
Glycemia (mmol/l)	6,2 ± 2,33	5,21±0,53	0,012
Insulinemia µUI/ml	12,93 ± 6,35	9,38 ±4,62	0,005
HOMA –IR index	3,6±2,28	2,16±1,07	0,001
IR*	27 (67,5%)	15(37,5%)	0,007
CT* (µmol/l)	3,76 ± 0,99	4,91±0,99	<0,001
HDL-CT * (µmol/l)	1,24 ± 0,33	1,45±0,38	0,012
TG* (µmol/l)	0,96 ± 0,29	1,07±0,5	0,215
MS * (%)	15 (37.5%)	6(15%)	0,02
CIMT ^{max} * (mm)	0,68 ± 0,16	0,60±0,13	0,002
CIMT^{75}*	33 (82,5%)	16 (40%)	< 0,001
HCV-RNA* UI/ml	3 697 572 ±6043422,57	-	-
Genotype 1/2	37/3	-	-
Stage of fibrosis			
Fo	3 (7,5%)		
F1	10(25%)	-	-
F2	12(30%)	-	-
F3	6(15%)		
F4	9(22,5%)		
Grade of inflammation			
A0	6 (15%)	-	
A1	13 (32,5%)	-	-
A2	12 (30%)	-	-
A3	6 (15%)	-	-
Not determined	3 (7,5%)		
Ultrasound steatosis	15 (37,5%)		

*CHC :chronic hepatitic C; *BMI :body mass index ;IR* :insulinoresistance ;HOMA* : homeostasis model assessment; CT* :cholesterol ; HDL* : high density lipoprotein acid ;TG* : triglycerides ; MS * :metabolic syndrom ; CIMT* :carotid intima-media thickness ;CIMT75 * :carotid intima-media thickness ≥ 75 percentiles ; HCV-RNA* : hepatitis C virus ribonucleic .

In a multi-variate study, only age ≥ 40 years and moderate-to-severe activity of hepatic impairment were independent factors associated with higher CIMT^{max} (p<0.001 and p=0.03 respectively).

Factors associated with infra clinic atherosclerosis with high cardiovascular risk

In univariate studies, the factors associated with the CIMT^{75} were HCV infection (p<0.0001), HOMA-IR index (p = 0.001), IR (p = 0.004) (Table 2). In multivariate analysis, only HCV infection was identified as an independent factor associated with CIMT^{75}(OR=4.81, CI :1,6-14,42, p=0,005) (Table 2).

DISCUSSION

In our study, it was shown that patients with chronic hepatitis C had a CIMT^{max} significantly higher compared to the control group. HCV infection has also been identified as the only independent factor associated with subclinical atherosclerosis with high cardiovascular risk.

Conflicting literature data have reported either normal (14-17) or increased CIMT (6,18-21), in patients with a clinical diagnosis of HCV infection compared with control populations.

Ishizaka et al were the first to report an association between carotid atherosclerosis and HCV infection, in a cohort of 1992 Japanese subjects. HCV-infected patients had a higher CIMT compared to non-infected subjects, as well as a significantly higher prevalence of carotid plaques (64% vs 25%, p<0.0001) (18).

These data were supported by a meta-analysis including nine case-control studies (22), chronic HCV infection was significantly associated with a higher CIMT compared to control subjects (p<0.001)

Other studies have noted a negative association between HCV infection and atherosclerosis. Most of them have included a small number of HCV-infected patients. Bilora et al followed the progression of atherosclerotic disease in 40 HCV-infected subjects and 40 controls for 5 years, both groups were matched for the cardiovascular risk factors and had a baseline comparable CIMT (14). A significant increase in CIMT was only observed in the control group.

Similarly, Tien et al found that HCV infection was associated neither with the CIMT nor with the presence of

Table 2. Univariate and multivariate analysis of risk factors associated with a high cardiovascular risk atherosclerosis

Variables	CIMT ^{75} (-)	CIMT ^{75} (+)	uni-variate study p	multi-variate study OR (CI 95%) p
HCV(+)	17,5%	82,5%	**<0.001**	4,81 (1,6-14,42)0,005
Mean age (years)	50,38±14,75	55,89±13,8	0,86	-
Age > 40 years	31,2%	43,7%	0,14	-
Gender	17,5%	20%	0,26	-
Diabetes	3,75%	8,75%	0,55	-
Arterial hypertension	12,5%	18,75%	0,90	-
Smoking	8,75%	11,25%	0,65	-
Obesity			0,27	-
CT *mmol/l	4,7±0,94	4,11±1,21	0.40	-
HDL-C* µmol/l	1,34±0,38	1,35±0,38	0.66	-
TG* µmol/l	0,97±0,46	1,03±0,38	0.50	-
Glycemia mmol/l	5,23±0,67	6,01±2,13	0.65	-
Insulinemia µUI/ml	8,71±3,66	12,7±6,39	0.43	-
HOMA-IR*	2,01±0,84	3,43±2,19	**0.001**	1,66 (0,71-3,84) 0,24
IR*	12,5%	40%	**0.004**	0,94 (0,15-5,97) 0,95
MS*	7,5%	18,75%	0.27	-
HCV-RNA* UI/l	3244375,2 ±4266499,65	3793704,6 ±640662377	0.83	-
Fibrosis ≥ F2	10%	22,5%	0,13	-

CIMT^{75} : carotid intima-media thickness>75 percentiles ; *OR :odds ratio ;*CI :confidence interval ; *HCV : hepatitic C virus; *CT :cholesterol ; *HDL : high density lipoprotein acid ;TG, triglycerides ; *HOMA, :homeostasis model assessment; *IR :insulinoresistance ; *MS :metabolic syndrom ;* HCV-RNA : hepatitis C virus ribonucleic .

carotid plaques (16). The same data were confirmed by an Egyptian study (17).

Few studies have evaluated the impact of fibrosis in chronic hepatitis C on the CIMT.They showed that severe fiborosis was associated with a higher risk of of carotid atherosclerosis (20,21).

In our work, significant fibrosis and moderate to severe activity were associated with a higher value of IMTon uni-variate study.

In the literature, no studies have directly evaluated the association between chronic HCV infection and subclinical atherosclerosis with high cardiovascular risk. The studies were rather focused on the impact of HCV infection on CVD mortality.

In the meta-analysis of Petta et al, HCV-infected patients had increased risks of CVD-related mortality compared with uninfected individuals (OR :1.65; CI 95%, 1.07-2.56; *p*=0 ,02)(22). In an American cohort inculding more than 10,000 HCV-infected blood donors, HCV infection was associated with an increased risk of cardiovascular mortality (OR=2.21, 95% CI: 1.41, 3.46) (23).

In a 16-year prospective cohort study, HCV infection was associated with increased hepatic and extra-hepatic mortality. CVD mortality was significantly higher in patients with detectable viral RNA (HR = 2.77, 95 % CI 1.49-5.15) (24).

The pathogenic mechanisms explaining the role of HCV in atherogenesis are not fully understood. Chronic hepatitis

C is associated with a complex condition promoting pro-atherogenic factors that are involved in endothelial dysfunction, insulin resistance, fibrosis and steatosis (9,25). These factors would be interconnected and driven mainly by dysregulation of several actors of innate and adaptive immunity (26,27).

Moreover, HCV RNA sequences have been detected in carotid plaques and in cerebral endothelium, suggesting a probable direct pro-atherogenic role of HCV (6,7,28).

The main limitation of our study lies in the small number of patients included and a selection bias in the recruitment of the control population which could affect the interpretation of results. The strength of our study lies in the fact that we did not only assess the CIMT as available data from literature but we were interested also in the evaluation of carotid atherosclerosis at high cardiovascular risk with a direct impact on cardiovascular disease .

CONCLUSION

Our findings suggest that the HVC was associated with an increased risk of Infra-clinical atherosclerosis with a high cardiovascular risk and we proposed that patients with chronic hepatitis C should receive periodic cardiovascular evaluation regardless of the presence of conventional cardiovascular risk factors.

Acknowledgements **:** No reported

REFERENCES

1. Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet Gastroenterol Hepatol 2017 ;2(3):161–76. https://doi.org/10.1016/S2468-1253(16)30181-9
2. Adinolfi LE, Restivo L, Zampino R, Lonardo A, Loria P. Metabolic alterations and chronic hepatitis C :treatment strategies. Expert Opin Pharmacother 2011 ;12(14):2215–34.
3. Zampino R, Marrone A, Restivo L et al. Chronic HCV infection and inflammation: Clinical impact on hepatic and extra-hepatic manifestations. World J Hepatol 2013;5(10):528.
4. Kukla M, Piotrowski D, Waluga M, Hartleb M. Insulin resistance and its consequences in chronic hepatitis C. Clin Exp Hepatol 2015;(1):17–29.
5. Adinolfi LE, Restivo L, Marrone A. The predictive value of steatosis in hepatitis C virus infection. Expert Rev Gastroenterol Hepatol 2013 ;7(3):205–13.
6. Boddi M, Abbate R, Chellini B et al. HCV infection facilitates asymptomatic carotid atherosclerosis: preliminary report of HCV RNA localization in human carotid plaques. Dig Liver Dis 2007 ;39 ; (1 Supp):55-60.
7. Boddi M, Abbate R, Chellini B et al. Hepatitis C virus RNA localization in human carotid plaques. J Clin Virol Off Publ Pan Am Soc Clin Virol 2010 ;47(1):72–5.
8. Stein JH, Korcarz CE, Hurst RT et al. Use of Carotid Ultrasound to Identify Subclinical Vascular Disease and Evaluate Cardiovascular Disease Risk: A Consensus Statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008 ;21(2):93–111.
9. Touboul P-J, Hennerici MG, Meairs S et al. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. Cerebrovasc Dis Basel Switz.2012;34(4):290-6.
10. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and betacell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28:412-419.
11. The International Diabetes Federation. Consensus Worldwide Definition of the Metabolic Syndrome [Internet].[updated 2017 Apr 5 ; cited 2020 jun 30].Available from https://www.idf.org/e-library/consensus-statements/60-idfconsensus-worldwide-definitionof-the-metabolic-syndrome
12. Boursier J, De Ledinghen V, Zarski J-P et al. Comparison of eight diagnostic algorithms for liver fibrosis in hepatitis C: new algorithms are more precise and entirely noninvasive. Hepatol Baltim Md 2012 ;55(1):58–67.
13. Denarié N, Gariepy J, Chironi G et al. Distribution of ultrasonographically-assessed dimensions of common carotid arteries in healthy adults of both sexes. Atherosclerosis 2000 ;148(2):297–302.
14. Bilora F, Campagnolo E, Rinaldi R, Rossato A, Arzenton M, Petrobelli F. Carotid and femoral atherosclerosis in chronic hepatitis C: a 5-year follow-up. Angiology 2008 ;59(6):717-20.
15. Caliskan Y, Oflaz H, Pusuroglu H et al. Hepatitis C virus infection in hemodialysis patients is not associated with insulin resistance, inflammation and atherosclerosis. Clin Nephrol 2009;71(2):147–57.
16. Tien PC, Schneider MF, Cole SR et al. Association of hepatitis C virus and HIV infection with subclinical atherosclerosis in the women's interagency HIV study. AIDS Lond Engl 2009 ;23(13):1781- 4.
17. Mostafa A, Mohamed MK, Saeed M et al. Hepatitis C infection and clearance: impact on atherosclerosis and cardiometabolic risk factors. Gut 2010 ;59(8):1135-40.

18. Ishizaka Y, Ishizaka N, Takahashi E et al. Association between hepatitis C virus core protein and carotid atherosclerosis. Circ J Off J Jpn Circ Soc 2003 ;67(1):26–30.

19. Targher G, Bertolini L, Padovani R, Rodella S, Arcaro G, Day C. Differences and similarities in early atherosclerosis between patients with non-alcoholic steatohepatitis and chronic hepatitis B and C. J Hepatol2007 ;46:1126–32.

20. Petta S, Torres D, Fazio G et al. Carotid atherosclerosis and chronic hepatitis C: a prospective study of risk associations. Hepatol 2012;55(5):1317–23.

21. Barakat AAE-K, Nasr FM, Metwaly AA, Morsy S, Eldamarawy M. Atherosclerosis in chronic hepatitis C virus patients with and without liver cirrhosis. Egypt Heart J 2017 ;69(2):139-47.

22. Petta S, Maida M, Macaluso FS et al. Hepatitis C Virus Infection Is Associated With Increased Cardiovascular Mortality: A Meta-Analysis of Observational Studies. Gastroenterology 2016 ;150(1):145-155.

23. Guiltinan AM, Kaidarova Z, Custer B et al. Increased all-cause, liver, and cardiac mortality among hepatitis C virus-seropositive blood donors. Am J Epidemiol 2008;167(6):743–50.

24. Lee M-H, Yang H-I, Lu S-N et al. Chronic hepatitis C virus infection increases mortality from hepatic and extrahepatic diseases: a community-based long-term prospective study. J Infect Dis 2012 ;206(4):469-77.

25. Roed T, Kristoffersen US, Knudsen A et al. Increased prevalence of coronary artery disease risk markers in patients with chronic hepatitis C – a cross-sectional study. Vasc Health Risk Manag 2014 ;10:55–62.

26. Yoon JC, Yang CM, Song Y, Lee JM. Natural killer cells in hepatitis C: Current progress. World J Gastroenterol 2016 ;22(4):1449–60.

27. Szereday L, Meggyes M, Halasz M, Szekeres-Bartho J, Par A, Par G. Immunological changes in different patient populations with chronic hepatitis C virus infection. World J Gastroenterol 2016 ;22(20):4848–59.

28. Fletcher NF, Wilson GK, Murray J et al. Hepatitis C virus infects the endothelial cells of the blood-brain barrier. Gastroenterology2012 ;142(3):634-43.