Original Article
Cent Asian J Med Sci. 2019 March;5(1):40-46. https://doi.org/10.24079/CAJMS.2019.03.006

Central Asian Journal of Medical Sciences
CAJMS

# Functional Abnormalities of the Liver in Diabetic Patients with and without Hepatitis C

**Altantuya Idkhuu[1], Badamjav Sevjid[1], Sainbileg Sonomtseren[1], Erdenetsogt Dungubat[1], Davaadorj Duger[1], Bira Namdag[1], Otgonbayar Radnaa[2]**

[1]Deparment of Internal Medicine, School of Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia; [2]General Hospital of Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia

**Submitted:** February 1, 2019
**Revised:** March 12, 2019
**Accepted:** March 15, 2019

**Corresponding Author**
Altantuya Idkhuu, MD, MSc
Department of Internal Medicine, School of Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar 14210, Mongolia
**Tel:** +976 99832614
**E-mail:**
altantuya.i@mnums.edu.mn



**Objective:** We aimed to compare liver function of diabetes mellitus patients with and without viral hepatitis C using the non-alcoholic fatty liver disease fibrosis score, aspartate transaminase to platelet ratio index, Fibrosis-4 Index, Mac-2-binding protein biomarker and ultrasonic liver stiffness measurements. **Methods:** The study was conducted based on convenience sampling of 123 patients. Slightly more than half of the study participants were male (53%, n=64). Thirty-three of the diabetics with hepatitis (mean age 52.31±9.8 years) and 90 diabetics without hepatitis (mean age 53.26±8.58) agreed to participate. Anthropometric measurements, non-alcoholic fatty liver disease fibrosis score, aspartate transaminase to platelet ratio index, Fibrosis-4 Index, Mac-2-binding protein biomarker, and ultrasonic transient elastography measurements were compared using independent t-tests for continuous variables and Wilcoxon rank sum tests for ordinal variables. **Results:** The median values of the Fibrosis-4 Index for those with and without hepatitis C were 1.3 vs. 0.9 ($p<.05$), Mac-2-binding protein biomarker 2.0 vs. 1.3 ($p<.0001$), ultrasonic liver stiffness measurements 10.3 vs. 6.9 ($p<.0001$), aspartate transaminase to platelet ratio 0.6 vs. 0.3 ($p<.001$), and Non-alcoholic fatty liver disease fibrosis scores were -0.2 vs. -0.9 ( $p<.004$), respectively **Conclusions:** Diabetic patients with hepatitis had statistically significantly higher Mac-2-binding protein biomarker, NAFLD Fibrosis Scores than patients without hepatitis. However, other fibrosis test results were similar in diabetic patients with hepatitis and without hepatitis C.

**Keywords:** Liver Fibrosis, Chronic Hepatitis, Non-alcoholic Fatty Liver Disease, Diabetes, Mongolia

## Introduction

Diabetic patients with viral hepatitis have a high risk of liver cirrhosis. Therefore, screening for fatty liver and liver fibrosis in diabetic patients is particularly important[1,2]. The main diagnostic method to stage fatty liver disease and liver fibrosis is liver biopsy and histology; however, it is also possible to detect differences in liver function using laboratory markers and determine the fibrosis stage of non-alcoholic fatty liver disease among patients who have type 2 diabetes mellitus[3].

Using a non-invasive method of determining liver fibrosis involves much research to discover new biomarkers and technologies to reveal liver fibrosis. Japanese researchers have found the Mac-2 binding protein glycan isomer (M2BPGi) to be a liver fibrosis glycol-biomarker with a unique fibrosis-related glycol-alteration. This biomarker helps to determine the stage of liver fibrosis in those with fatty liver disease and hepatitis C (HCV)[4].

Research regarding liver fibrosis tests have been done in many countries in the world and the use of new biomarkers and other tests has been increasing too. Researcher D. Khishgee has compared aspartate transaminase to platelet ratio index (APRI) and Fibrosis-4 Index (FIB-4) to blood coagulation changes in Third Central Hospital of Mongolia[5]. But to our knowledge, there has been no published research comparing non-alcoholic fatty liver disease fibrosis score (NAFLD Fibrosis Score), M2BPGi biomarker, and transient ultrasonic liver stiffness in diabetic patients in Mongolia.

We aimed to compare the liver function of diabetes mellitus patients with and without HCV using the NAFLD Fibrosis Score, APRI, FIB-4 index, M2BPGi biomarker, and transient ultrasonic liver stiffness.

## Materials and Methods

### Study subjects

The study population was 120 of 423 diabetic patients at MNUMS General Hospital Ambulatory Clinic willing to participate in the study. Thirty-three diabetic patients with HCV (mean age 52.31±9.8 years) and 90 without HCV (mean age 53.26±8.58) agreed to participate.

### Clinical and biochemical assessment

Relevant clinical data were recorded, including the patient's age, sex, weight, and height. Body mass index (BMI) was calculated. Blood specimens were obtained at the MNUMS General Hospital Ambulatory Clinic. The blood samples were stored at -80° C until analysis.

The blood was thawed, and tests were performed using a conventional automated analyzer. The platelet count, prothrombin time, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase, albumin, cholesterol, triglyceride, and fasting plasma glucose were measured. The APRI, FIB-4 index, and the NAFLD fibrosis score were calculated[5]. Liver stiffness measurements were made using a FibroScan® 502 Touch ultrasonographic elastography machine (Echosens, France). This device works by measuring the velocity of a 50-MHz wave through the liver which is then be converted into liver stiffness, expressed as a continuous variable in kilopascals.

### Measurements

The patients were categorized into two groups, diabetic patients with HCV or diabetic patients without HCV. The variables compared were NAFLD Fibrosis Score, APRI, FIB-4 Index, Mac-2-binding protein biomarker and FibroScan® measurements. All laboratory results were treated as continuous data. Covariates were demographic characteristics, such as age, gender, and anthropometric measurements. The literature was reviewed to identify cut off values for each of the tests according to the METAVIR classification (F0 indicating no fibrosis; F1, enlarged, fibrotic portal tracts; F2, periportal or portal–portal septa but intact architecture; F3, fibrosis with architectural distortion but no obvious cirrhosis; and F4, probable or definite cirrhosis)[6]. From the literature, the M2BPGi cut-off values used were 0.57, 0.7, 1.02, 1.57, and 2.96 for each of the METAVIR stages respectively while the FIB-4 cut-off value of 0.725 (0.659-0.791), and APRI cut-off value of 0.681 (0.613-0.749) were used[2,7,8]. According to the 3 subgroups of low, intermediate and high probability of fibrosis, we used parameters of NAFLD Fibrosis Score $< -1.5$ for low, NAFLD Fibrosis Score of -1.5 to 0.67 for intermediate and NAFLD Fibrosis Score $\geq 0.67$ for high probability of fibrosis[9].

### Statistical analysis

The normality of each continuous variable was checked with

the Kolmogorov-Smirnov test. Independent t-tests were used to compare the results for normally distributed variables for patients with and without HCV. When the Kolmogorov-Smirnov testing of the independent variables indicated the results were not normally distributed, nonparametric tests were used.

Categorical data were analyzed using the Chi-Square test. A p-value was considered significant when p<.05. Statistical analyses were conducted using SPSS version 23.0 software.

### Ethical statements

The Institutional Review Board of Ministry of Health of Mongolia approved the study design (Protocol #09 on 29th September 2015), and written informed consent was obtained for all patients.

## Results

Table 1 shows the socio-demographic characteristics of the study population. There was no difference in gender between the groups or ages of the patients except for patients in the oldest category where there were more patients without HCV infection in patients 61-70 years of age (p<.02).

Table 2 shows the anthropometric data of the study patients, divided according to the presence of HCV. Among those with HCV, the waist to hip circumference ratio was statistically significantly different than those without HCV.

The mean age of all the patients was 52.9±8.65 years, their average height was 166.4±9.66 cm, average weight 84.2±16.5 kg, average waist circumference 95.88±13.8 cm and average body fat 36.9±7 percent, and there were no statistically significant differences between these measurements in with HCV and compared to those without HCV infection. However, their waist to hip circumference ratios were significantly different (0.99±0.1 vs. 0.92±0.4, p=.005).

Table 3 shows the results of the noninvasive liver fibrosis tests for patients with and without HCV infection.

All of the testing methods resulted in statistically differences comparing diabetic patients with and without HCV. Using the cut off intervals provided from the literature, the M2BPGi and FibroScan® tests were least likely to conclude that the measurements for the two groups were different when in fact they were not (p<.0001), followed by APRI (p<.001) and NAFLD Fibrosis Scores (p<.004). Although the risks of this error were low for FIB-4 measurements (p<.05), this error was most likely using this test, decreasing its diagnostic utility. The average M2BPGi for patients with HCV was higher than patients without it by

**Table 1.** The socio-demographic characteristics of diabetes mellitus patients with and without chronic HCV (N=120)

| | Without HCV N=90 N (%) | With HCV N=30 N (%) | a$\chi$2 | p-value |
|---|---|---|---|---|
| Gender | | | | |
| Female | 39 (43.3) | 17 (56.7) | 1.607 | .29 |
| Male | 51 (56.7) | 13 (43.3) | | |
| Age | | | | |
| 31-40 | 12 (13.3) | 2 (6.7) | 1.579 | .49 |
| 41-50 | 23 (25.6) | 7 (23.3) | | .47 |
| 51-60 | 38 (42.2) | 16 (53.3) | | .09 |
| 61-70 | 17 (18.9) | 5 (16.7) | | .02 |
| Location | | | | |
| Urban | 65 (72.2) | 21 (70.0) | 4.204 | .50 |
| Rural | 25 (27.8) | 9 (30.0) | | |
| Total | 120 (100) | 30 (100) | | |

[a]$\chi^2$- Chi-Square Test

**Table 2.** Comparison between anthropometric measurements between diabetic patients with and without HCV.

| | Without HCV N=90 | With HCV N=33 | p-value[c] |
|---|---|---|---|
| | Mean±SD | Mean±SD | |
| Age (years) | 52.4±9.8 | 53.4±7.5 | .626 |
| Height (cm) | 164.5±10.8 | 168.3±8.6 | .381 |
| Weight (kg) | 83.1±17.7 | 85.3±15.3 | .421 |
| BMI (kg/m$^2$) | 30.14±4.6 | 30.2±4.5 | .267 |
| WC[a] (cm) | 94.96±13.1 | 96.8±12.9 | .399 |
| HC[b] (cm) | 102.4±15.1 | 103.3±11.0 | .158 |
| WC/HC ratio | 0.99±0.1 | 0.92±0.4 | .005 |
| Body fat (%) | 38.4±6.8 | 35.6±7.5 | .065 |

[a]Waist circumference. [b]Hip circumference. [c]Calculated using independent t-tests

**Table 3.** Comparison between liver fibrosis tests in diabetic patients with and without HCV.

| | Without HCV N=90 | With HCV N=30 | p-value | 95% CI |
|---|---|---|---|---|
| | Mean (SD) | Mean (SD) | | |
| M2BPGi (COI) | 1.3±0.7 | 2.0±1.2 | <.0001 | [-1.1;-0.4] |
| FibroScan® (kPa) | 6.9±1.7 | 10.3±6.1 | <.0001 | [-4.8;-2.0] |
| FIB-4 (COI) | 0.9±0.8 | 1.3±0.9 | .05 | [-0.7-0.01] |
| APRI (COI) | 0.3±0.2 | 0.6±0.6 | .001 | [-0.4;-0.1] |
| NAFLD Fibrosis Score (COI) | -0.9±1.2 | -0.2±1.2 | .004 | [-1.2;-0.3] |

Test of Normality was checked with Kolmogorov-Smirnov. Calculates with Independent Sample T test with p<.05 considered significant; **p<.01 ;95% CI: Confidence Interval.

0.7 COI (0.47–12.9 COI). Furthermore, the average FibroScan® result was 3.4 kPa higher in patients with HCV (p<.0001), FIB-4 was 0.4 COI higher (p=.05), APRI was .3 COI higher (p=.001), and NAFLD Fibrosis Score was 0.7 COI higher (p=.004) than the HCV negative group.

Figure 1 shows liver fibrosis stages of patients without HCV using the cutoff values published by other investigators to determine their METAIVR classification. Using the by M2BPGi biomarker, 5.6% of the participants were diagnosed in stage F0, 10.0% of them were F1 stage, 27.8% were F2, 31.1% m were F3 and 25.6% were in stage F4. By FibroScan® 24.4% of the patients were diagnosed with the F0 stage, 46.7% were F1, 27.8% were F2, 1.1% were in F3 stage and none were in stage F4. Using the FIB-4 test, 86.7% of the patients were in F0 stage, 6.7% were in F1, 1.1% were F2, and 3.3% were in both F3 and F4 stages. By the APRI test result, 92.2% of the patients were diagnosed in F0 stage, 6.7% were in F1, 1.1% were F2 and none were in F3 or F4 stages. By the NAFLD-Fibrosis Score, 18.9% of the patients were in F0 stage, 11.1% of them were in F1, 52.2% were F2, 12.2% were in F3, and 5.6% of them were in stage F4.

Figure 2 shows liver fibrosis stages of patients with HCV infection according to METAIVR classification. Using the M2BPGi biomarker, no patients were diagnosed in F0 and F1 stages, 23.3% of them were in F2, 26.7% were F3 and 50% of them were in F4 stage. By FibroScan®, 23.3% of the patients were diagnosed in F0 stage, 33.3% of them were in F1 and F2 stages, 6.7% were in F3 stage and 3.3% were in F4 stage. By the FIB-4 test, 50.0% of the patients were in F0 stage, 33.3% were in F1 and F2 stages and 3.3% in both F3 and F4 stages. Using the APRI test result, 46.7% of the patients were diagnosed in F0 stage, 40% of them were in F1 stage, 3.3% of them were in F2 stage and there were no patients in stage F3 but 10% of them were in stage F4. By the NAFLD Fibrosis Score, 13.3% of the

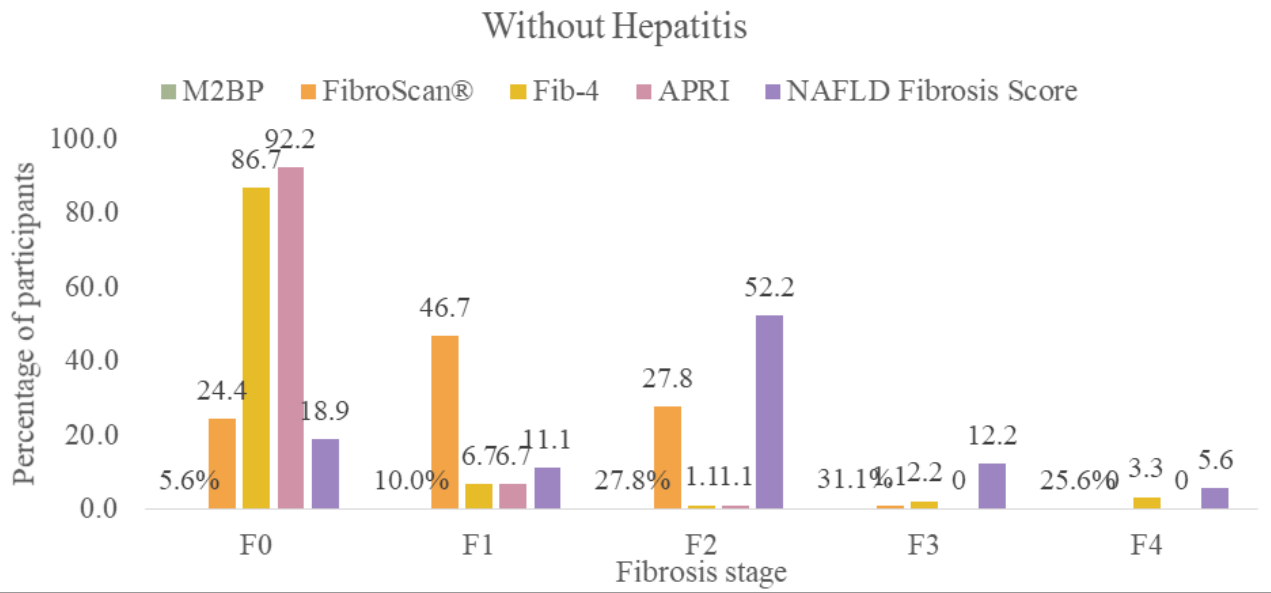


**Figure 1.** Comparison of fibrosis stages using non-invasive fibrosis tests in diabetic patients without HCV.

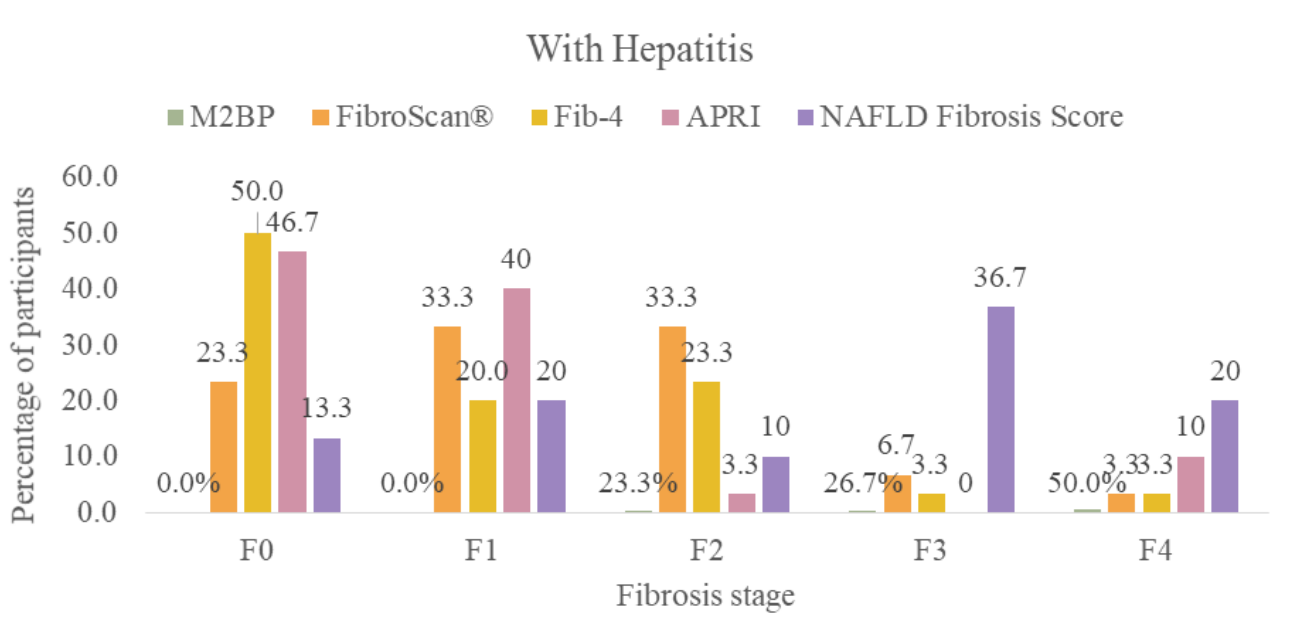


**Figure 2.** Comparison of fibrosis stages using non-invasive fibrosis tests in diabetic patients with HCV.

patients were categorized into stage F0, 20.0% of them were in F1 stage, 10.0% were in F2, 36.7% were in F3 and 20.0% of them were in stage F4.

## Discussion

Our study showed that liver fibrosis stage was most closely associated with serum M2BPGi level. In addition, the degree of necroinflammation had no apparent effect on the M2BPGi value. Based on these results, we proposed a clinical management algorithm using an M2BPGi assay to predict the fibrosis stage in diabetic patients. This approach could be used reliably for the first-line pre-therapeutic evaluation of fibrosis in hepatitis-infected diabetic patients. On the other hand, the most widely

used noninvasive techniques have recently shifted to physical measurements, such as FibroScan®, 27-30 acoustic radiation force impulse, and real-time strain elastography. FibroScan® has the advantages of being rapid and technically simple; however, operator skill affects its diagnostic accurracy[10].

Our non-invasive liver fibrosis test results average of diabetic patients without HCV for M2BPGi is 1.3±0.7 (F1-F2, in FibroScan® is 6.9±1.7 kPa (F0-F1), for FIB-4 is 0.9±0.8 for COI is (F0-F1), for APRI is .3±.2 COI (F0-F1), for NAFLD Fibrosis Score is -.9±1.2 COI (F0-F1) these results are similar to Yung-Yu's research results[11]. Liver fibrosis test average of diabetic patients with HCV for M2BPGi is 2.0±1.2 (F3-F4), by FibroScan® 10.3±6 M2BPGi.1 kPa (F3-F4), FIB-4 1.3±.9 COI (F2-F3), APRI .6±.6 COI (F2-F3), NAFLD Fibrosis Score -.2±1.2 COI (F3-F4). These results are similar to the research of Toshima et al[12]. The results indicate that the fibrosis stage was higher in diabetic patients with HCV indicating that patients with both diabetes and HCV have more liver fibrosis and these results have been verified by Boursier et al[13].

Diabetes is intimately related to hepatitis infection. Numerous studies in animal models and humans report an increased prevalence of type 2 diabetes among hepatitis patients. However, the underlying mechanisms are only partly understood, though recent data suggest a direct inhibitory effect of the virus on the insulin signaling pathway. An increase of fasting glucose and a decrease in insulin sensitivity has been observed in hepatitis-infected subjects with a moderate or severe degree of hepatic fibrosis[14,15].

Diabetes has been shown in several, but not all, studies to have a deleterious effect on the clinical course of chronic hepatitis infection, and the inconsistency may be explained by differences in the baseline characteristics of the patients[16-18]. Small studies suggest that lifestyle intervention and metformin may increase the sustained virologic response rate, but further studies are needed to confirm these findings. The effect of type 2 diabetes mellitus on the direct-acting anti-viral treatment drugs is still unclear[19].

The analysis of correlation of M2BPGi with Brunt stages, which are used as indices of severity of fibrosis in liver biopsy features, showed potential usefulness of this novel marker in identifying F3 (bridging fibrosis) or higher stage cases[20].

Our study has several limitations. The use of the M2BPGi values for monitoring the natural history, predicting outcomes, and predicting responses to therapeutic interventions remain unknown. In fact, the prevalence of non-alcoholic fatty liver disease is high among individuals with diabetic dyslipidemia, and some patients have already managed their condition through lifestyle interventions and/or medication at the time of liver biopsy[21]. Further research should include diabetic patients who have hepatitis to compare liver biopsy staging to liver fibrosis markers. Also, the impact of the duration of diabetes on liver fibrosis should be compared to healthy people. Further prospective studies are necessary to address these issues.

**Conclusions**

Diabetic patients infected with HCV had higher M2BPGi, NAFLD Fibrosis Score than patients without HCV. However, the results of the other fibrosis test results were similar in diabetic patients with HCV and without HCV.

## Conflict of Interest

The authors declare that they have no competing interests.

## Acknowledgments

We would like to thank the staff at the Mongolian Representative Office of the Sysmex Corporation (Japan) and Proliance LLC, for their help in completing this research project. We would also like to thank Prof. R. Otgonbayar, Prof. N. Bira, E. Uranbaigal, and B. Ariunaa for their effort in this research project.

## References

1. Altaisaikhan KH. Clinical Practice Guidelines for the Millennium Challenge Health Project. Ulaanbaatar, Mongolia: Monkhiin useg; 2011. p 15-18.
2. Toshima T. A novel serum marker, glycosylated Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA(+)-M2BP), for assessing liver fibrosis. J Gastroenterol 2015; 50: 76-84.
3. Angulo P. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. J Hepatol 2007; 45: 846-54.
4. Masanori A. Association between Wisteria floribunda

agglutinin-positive Mac-2 binding protein and the fibrosis stage of non-alcoholic fatty liver disease. J Gastroenterol 2014; 13: 303-10.
5. Khishgee D. Liver fibrosis stage to compared portal hypertension and coagulopathy [dissertation]. Ulaanbaatar, Mongolia: Mongolia National University of Medical Sciences; 2017.
6. Bedosa P. The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. J Hepatol 1994; 20: 15–20.
7. Xianghua Z. Performance of several simple, noninvasive models for assessing significant liver fibrosis in patients with chronic hepatitis B. Croat Med J 2015; 56: 272–9.
8. Sombat T. NAFLD fibrosis score: A prognostic predictor for mortality and liver complications among NAFLD patients. World J Gastroenterol 2013; 19: 1219-29.
9. Hui J. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression. J Gastroenterol 2003; 125: 1695 –704.
10. Koyama N. Assessment of hepatic fibrosis with superb microvascular imaging in hepatitis C virus-associated chronic liver diseases. Hepatol Res 2017; 47: 593-7.
11. Yung-Yu H. Routine blood tests to predict liver fibrosis in chronic hepatitis C. World J Gastroenterol 2012; 18: 746-53.
12. Toshima T. A novel serum marker, glycosylated Wisteria floribunda agglutinin-positive Mac-2 binding protein, for assessing liver fibrosis. J Gastroenterol 2015; 50: 76-84.
13. Boursier J. Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease. J Hepatol 2016; 65: 570-8.
14. Kazumi Y. Elevated Serum Levels of Wisteria floribunda Agglutinin-Positive Human Mac-2 Binding Protein Predict the Development of Hepatocellular Carcinoma in Hepatitis C Patients. J Hepatol 2014; 60: 1563–70.
15. Knobler H. Hepatitis C and insulin action: An intimate relationship. World J Hepatol 2016; 8: 131-8.
16. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002; 346: 1221-31.
17. Levinthal G. Liver disease and diabetes mellitus. Clin Diabetes 1999; 17: 73.
18. Lin ZH. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. J Hepatol 2011; 53: 726-36.
19. Larter CZ. Insulin resistance, adiponectin, cytokines in NASH: Which is the best target to treat J Hepatol 2006; 44: 253–61. doi: 10.1016/j.jhep.2005.11.030.
20. Bugianesi E. Insulin resistance: A metabolic pathway to chronic liver disease. J Hepatol 2005; 42: 987–1000.
21. Porepa L. Newly diagnosed diabetes mellitus as a risk factor for serious liver disease. CMAJ 2010; 182: E526–31.