Research article

A comparative study of cardiovascular autonomic function in patients with alcoholic-fatty liver disease and non-alcoholic fatty liver disease

Laishram Sureiya Devi[1], Rolinda Rajkumari[1], Susie Keithellakpam[1], Joshna Thiyam[1], Kh. Lokeshwar Singh[2], Ngamba Akham[3]

[1]Department of Physiology, [2]Department of Medicine, [3]Community Medicine Department, Jawaharlal Nehru Institute of Medical Sciences, Porompat, Imphal-East, 795005, Manipur, India

(Received: January 2021 Revised: December 2021 Accepted: January 2022)

Corresponding author: **Rolinda Rajkumari.** Email: rolinda_rk65@rediffmail.com

ABSTRACT

Introduction and Aim: Autonomic dysfunction is found in chronic liver disease, and presence of it increases the risk of mortality. High level of liver enzymes predicts liver disease and cardiovascular event. The present study was conducted to compare the cardiovascular autonomic function between patients with alcoholic-fatty liver disease (AFLD) and non-alcoholic fatty liver disease (NAFLD) using heart rate variability (HRV) indices and to see if any correlation exists between HRV and liver enzymes.

Materials and Methods: Study population included 60 patients with fatty liver disease (30 AFLD and 30 NAFLD) and 30 healthy controls. HRV analysis was done using the Lab Chart Prov8.1.13 with HRV module version 2.0. Time-domain and frequency-domain were estimated. Statistical analysis was done by using Student's t test and Spearman's rho correlation test.

Results: The SDRR, LF and HF were significantly decreased in patients with fatty liver disease when compared to controls. The SDRR, RMSSD, LF, HF and LF/HF ratio of patients with AFLD when compared to patients with NAFLD showed no statistically significant differences. There was a negative significant correlation between the value of GGT with SDRR, LF and HF in patients with AFLD.

Conclusion: Fatty liver disease is associated with reduced HRV. There is no significant difference in HRV indices between patients with AFLD and NAFLD. The greater the liver disease severity, the greater is the HRV impairment. Hence, assessment of autonomic function should be given due importance in fatty liver disease.

Keywords: Heart rate variability; fatty liver disease; Gamma-glutamyltransferase; autonomic function.

INTRODUCTION

Chronic liver disease (CLD) is a major public health issue in the world. Globally, it is shown that 844 million people are having chronic liver disease with a mortality rate of 2 million deaths per year (1). The estimated worldwide prevalence of alcoholic liver disease (ALD) is 8.5% with the highest prevalence (around 12%) observed in Europe and the United States while that of non-alcoholic fatty liver disease (NAFLD) is 25% with the highest prevalence (17% to 46%) found in western countries (2,3).

Chronic liver disease is associated with various haemo-circulatory disturbances such as impairment of cardiovascular autonomic reactivity which is characterized with resting tachycardia, relative hypotension, and diminished vascular resistance (4). Negru *et al.*, (5) evaluate autonomic dysfunction by using 24-hour ECG monitoring of heart rate variability (HRV) parameters in liver cirrhosis patients and concluded that liver cirrhosis was associated with reduced HRV parameters. They also observed lowest HRV value in alcoholic liver cirrhosis patients. Another study by Liu *et al.*, (6) demonstrated that non-alcoholic fatty liver disease was associated with autonomic changes evaluated by heart rate variability (HRV) indices. All the HRV indices were significantly decreased in NAFLD (6). Previous studies have shown that the prevalence of autonomic dysfunction was in comparable frequency between alcoholic-related and non-alcoholic related liver disease when assessed using cardiovascular reflex tests (7-9). Additionally, Fleckenstien *et al.*, (7) reported presence of autonomic dysfunction in liver disease increases the risk of mortality by 5-fold.

Liver enzymes such as γ-glutamyltransferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were elevated in plasma and are used as markers of hepatic dysfunction due to numerous disease conditions including non-alcoholic fatty liver disease (NAFLD; 10). In a cohort study by Lee *et al.*, (11) elevated aminotransferase level was found associated with liver, cardiovascular and all-cause mortality. Fraser *et al.*, (12) also reported a

meta-analysis exploring the association of GGT and ALT with incident CHD, stroke, and a combined outcomes in the British Women's Heart and Health study. They found that GGT but not ALT was found associated with the incident cardiovascular events independently of alcohol intake.

Previous studies on autonomic function were done in patients with cirrhotic liver disease and most of the comparison between alcoholic-related and non-alcoholic related were using Classic cardiovascular reflex tests. But the present study was conducted to compare the autonomic function between patients with alcoholic fatty liver disease (AFLD) and NAFLD by using short-term HRV and also to see if any correlation exists between HRV indices and liver enzymes (ALT, AST and GGT).

MATERIALS AND METHODS

The study was conducted on 60 patients with fatty liver disease (30 alcoholic fatty liver disease and 30 non-alcoholic fatty liver disease) with age ranged from 31-50 years and were recruited from Out-patient Department of Medicine, Jawaharlal Nehru Institute of Medical Sciences, JNIMS. There were 7 males and 23 females in patients with NAFLD. In alcoholic fatty liver disease, only males were present since alcoholic females do not attend OPD due to social stigma/ culture. Inclusion criteria for AFLD included patients who had a significant history of alcohol consumption exceeding 210g/week in males and 140gm/week in females for the last 2 year and ultrasound showing fatty liver whereas patients with no history of alcohol consumption and ultrasound showing fatty liver were taken for NAFLD. Exclusion criteria included patients with history of hepatitis, diabetes mellitus, thyroid disorders, heart disease or who were using drugs which can affect HRV (eg., phenytoin, amiodarone, propranolol, methyldopa etc.,).

Thirty healthy controls (11 males and 19 females; aged from 32-48 years) were recruited from the staff of the Institute. Controls were healthy as reported by history, physical examination, and none of them had consumed alcohol or were on medications (self-reported).

The study was carried out after taking clearance from the Institutional Ethics Committee (IEC number: 112(5)) of JNIMS. Prior to participation for the study, the purpose of the study was explained to all the subjects and informed written consent was taken.

Liver function test analysis

Venous blood samples for all the patients were taken in the morning after an overnight fast of more than 10 hours. Total bilirubin (Azobilirunin/dyphyline method), direct bilirubin (Dual WL Spectophotometric method), indirect bilirubin (Calculated), AST (Kinetic with Pyridoxal-5 phosphate method), ALT ((Kinetic with Pyridoxal-5 phosphate method), GGT (LY-Glutamyl 3 Carboxy 4 Nitroanilide Substrate method), Alkaline phosphatase (ALP) (pNNP/AMP buffer method), total protein (Biuret; alkaline cupric sulphate method), albumin (Bromocresol green dye binding method), globulin (Calculated) levels were estimated.

All the subjects were asked to come to Autonomic Laboratory, Department of Physiology, JNIMS. Body weight and height were assessed by using a standardized weighing machine and height scale. Body mass index (BMI) was calculated by Quetlet index: Wt (Kg) / Ht (m^2). The recording of heart rate variability was done between 9:00 am and 12.00 noon in a controlled ambient temperature of 23º to 25◦C. The subject was asked to lie down on the couch where electrodes for lead II ECG acquisition by Labchart Prov8.1.13 (AD Instruments, Australia) were attached. A 5-minute lead II ECG (sampling rate 1KHz) was recorded after taking rest for 10 minutes. The subjects were instructed to breathe regularly and calmly with normal breathing rate of 12-16 breaths per minute and stay awake to prevent artefacts in the recording. The ECG signal was kept by data acquisition software (Lab Chart Prov8.1.13) with HRV module version 2.0; (AD Instruments, Australia) using PowerLab 26T (AD Instruments, Australia). “R” waves were detected, and artefact-free signals were kept. Then, time-domain and frequency-domain components of HRV were analyzed using Lomb Scargle Periodogram. Time domain components of HRV are SDRR (standard deviation of interbeat interval for all sinus beats) and RMSSD (square root of the mean squares differences between adjacent RR intervals) which denote overall HRV and vagal activity respectively. Under frequency-domain, low frequency (LF: 0.04–0.15Hz) and high frequency (HF: 0.15–0.40Hz) power in absolute values of power (ms^2) and LF/HF ratio were calculated. The high frequency power denotes parasympathetic activity, low frequency denotes combination of sympathetic and parasympathetic input while LF/HF ratio indicates sympathovagal balance.

Statistical analysis

The data were entered in MS Excel and Statistical Package for Social Sciences (SPSS) version 23. The statistical analysis was done by applying descriptive statistics i.e., mean ± S.D. Comparison of HRV indices between patients with AFLD and NAFLD was done by using Student’s t test. Correlation of HRV indices and liver enzymes (AST, ALT, GGT) in patients with AFLD and NAFLD was made by using Spearman’s rho correlation test and ‘p’ value of less than 0.05 was considered significant.

RESULTS

Table 1 shows the demographic profile of controls and patients with fatty liver disease. The mean age, weight, height and BMI showed no significant differences between controls and patients with AFLD and also with NAFLD. There was no significant difference in demographic profile between patients with AFLD and NAFLD.

Table 2 shows the distribution of liver function test of all the subjects. The mean values of liver enzymes were significantly higher in both patients with fatty liver disease (AFLD and NAFLD) when compared with the controls. However, there were no significant differences between the patients with AFLD and NAFLD.

Table 1: Demographic profile of all subjects

Variables	Controls (n=30)	AFLD (n=30)	NAFLD (n=30)
Age (years)	41.16 ± 3.7	43.36 ± 5.24	43.26 ± 5.29
Weight (Kg)	65.03 ± 9.51	64.67 ± 9.86	65.10 ± 8.91
Height (cm)	158.76 ± 9.22	157.16 ± 4.28	155.23 ± 4.31
BMI (kg/m^2)	25.91 ± 3.01	26.15 ± 3.65	26.99 ± 3.37

n: number of patients; BMI: Body mass index.

Table 2: Distribution of liver function test of all subjects.

Variables	Controls (n=30)	AFLD (n=30)	NAFLD (n=30)
Total protein (g/dl)	7.33 ± 0.63	7.08 ± 0.48	7.28 ± 0.37
Albumin (g/dl)	3.90 ± 0.42	3.76 ± 0.2	3.86 ± 0.25
Globulin (g/dl)	3.43 ± 0.45	3.29 ± 0.28	3.42 ± 0.21
A/G ratio	1.12 ± 0.19	1.14 ± 0.09	1.13 ± 0.10
AST (unit/L)	25.96 ± 5.89	55 ± 29.5*	43.4 ± 22.79$^{\#}$
ALT (unit/L)	29.86 ± 16.58	50.56 ± 27.52*	58.23 ± 33.30$^{\#}$
GGT (unit/L)	30.93 ± 16.43	67.33 ± 27.83*	43.93 ± 10.82$^{\#}$
Total bilirubin (mg/dl)	0.76 ± 0.25	0.81 ± 0.24	0.78 ± 0.21
Indirect bilirubin (mg/dl)	0.51 ± 0.17	0.47 ± 0.12	0.46 ± 0.15
Direct bilirubin (mg/dl)	0.25 ± 0.16	0.33 ± 0.21	0.31 ± 0.09
Alkaline phosphatase (unit/L)	136.8 ± 54.37	168.66 ± 50.72*	161.33 ± 36.78$^{\#}$

n: number of patients; p*< 0.05: controls and AFLD; p$^{\#}$< 0.05: controls and NAFLD;
A/G: Albumin /globulin ratio; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase;
GGT: Gamma-glutamyltransferase.

Table 3: Basal blood pressure and basal heart rate of all subjects.

Variables	Controls (n=30)	AFLD (n=30)	NAFLD (n=30)
SBP (mmHg)	125.80 ± 7.98	127.53 ± 11.08	123.56 ± 10.52
DBP (mmHg)	81.6 ± 3.90	84.43 ± 8.50	81.43 ± 8.44
HR (b/m)	80.5 ± 6.88	74.49 ± 10.11	76.57 ± 8.86

n: number of patients; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart rate; b/m: beats/minute.

Table 4: Comparison of time domain and frequency domain of HRV in Controls vs patients with AFLD, controls vs patients with NAFLD and patients with AFLD vs patients with NAFLD

Variables	Controls (n=30)	AFLD (n=30)	NAFLD (n=30)
LF (ms^2)	439.2 ± 305.74	266.1 ± 157.6 *	312.7 ± 142.28$^{\#}$
HF (ms^2)	276.4 ± 217.04	175.7 ± 163.57*	187.24 ± 86.06$^{\#}$
LF/HF ratio	1.78 ± 0.76	1.82 ± 0.67	1.95 ± 1.31
SDRR (ms^2)	36.73 ± 11.58	27.55 ± 7.09*	25.58 ± 5.08$^{\#}$
RMSSD (ms^2)	23.88 ± 7.70	21.07 ± 7.42	21.67 ± 8.74

n: number of patients; p*< 0.05: controls and AFLD; p$^{\#}$< 0.05: controls and NAFLD; LF: low frequency; HF: high frequency; LF/HF: ratio of low frequency to high frequency; SDRR: standard deviation of interbeat intervals for all sinus beats; RMSSD: square root of the mean square's differences between adjacent RR intervals.

Table 3 shows basal blood pressure and basal heart rate of controls and patients with fatty liver disease. The basal BP and basal HR showed no statistically significant differences between the controls and patients with fatty liver disease and also between patients with AFLD and NAFLD.

Table 4 shows comparison of HRV indices between the controls and patients with fatty liver disease. The mean values of SDRR, LF and HF were significantly decreased in both patients with AFLD and NAFLD compared with the controls. However, the mean values of HRV indices of patients with AFLD when compared to patients with NAFLD showed no statistically significant differences.

Table 5 shows correlation between the HRV indices and liver enzymes (ALT, AST, GGT) in patients with AFLD patients. There was a negative significant correlation between the value of GGT with SDRR, LF and HF in patients with AFLD.

Table 6 shows correlation between the HRV indices and liver enzymes (ALT, AST, GGT) in patients with NAFLD. No statistically significant correlation was observed.

Table 5: Correlation between the HRV indices and liver enzymes in patients with AFLD

Variables	AST		ALT		GGT	
	Correlation coefficient (r)	p-value	Correlation coefficient (r)	p-value	Correlation coefficient (r)	p-value
LF	-0.043	0.821	-0.111	0.557	-0.374	0.042*
HF	-0.235	0.212	-0.159	0.402	-0.384	0.036*
LF/HF	0.329	0.076	0.132	0.487	0.294	0.15
SDRR	-0.209	0.269	-0.112	0.557	-0.554	0.002*
RMSSD	0.099	0.602	-0.095	0.616	-0.271	0.148

p* < 0.05; LF: low frequency; HF: high frequency; LF/HF: ratio of low frequency to high frequency; SDRR: standard deviation of interbeat intervals for all sinus beats; RMSSD: square root of the mean square's differences between adjacent RR intervals; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyltransferase.

Table 6: Correlation between the HRV indices and liver enzymes in patients with NAFLD

Variables	AST		ALT		GGT	
	Correlation coefficient (r)	p-value	Correlation coefficient (r)	p-value	Correlation coefficient(r)	p-value
LF	-0.142	0.45	-0.0820	0.66	0.008	0.96
HF	-0.294	0.115	-0.292	0.117	-0.179	0.344
LF/HF	0.269	0.151	0.306	0.100	0.262	0.162
SDRR	-0.266	0.155	-0.076	0.688	-0.131	0.489
RMSSD	-0.162	0.391	-0.070	0.715	-0.055	0.772

LF: low frequency; HF: high frequency; LF/HF: ratio of low frequency to high frequency; SDRR: standard deviation of interbeat intervals for all sinus beats; RMSSD: square root of the mean square's differences between adjacent RR intervals; AST: Aspartate aminotransferase. ALT: Alanine aminotransferase; GGT: Gamma-glutamyltransferase.

DISCUSSION

In the present study, the time domain, SDRR, which is the indicator of overall function of autonomic activity was reduced in both the patients with AFLD and NAFLD as compared to controls. It was also observed that there was significantly reduced LF and HF of frequency domain of HRV in both patients with AFLD and NAFLD, when compared with the controls except for LF/HF ratio suggesting impairment of parasympathetic and sympathetic activity. Similar findings were reported by Coelho *et al.,* (13) which showed a severe reduction of SDNN and pNN50 in cirrhotic patients when compared to healthy volunteers. It also observed a marked decrease in average total power, with reduction of all components (VLF, LF, HF) except for LF/HF ratio (13). Similarly, a significant decrease in LF and HF in patients with liver cirrhosis when compared to normal group has been reported. However, the LF/HF ratio was found to be increased in the patients with liver cirrhosis (14).

Milovanovic et al., (15) reported that 16 out of 25 patients with alcoholic cirrhosis admitted to Department of Gastroenterology of Clinical and Hospital Center "Bezanijska Kosa" had at least one or more abnormal parasympathetic function test. All the spectral components (VLF, LF, HF) detected by short-term spectral analysis were significantly lower when compared to controls (15). Similar results were also reported in patients with NAFLD. Evaluation of autonomic function by HRV index in patients with NAFLD showed that all the HRV indices were significantly decreased in NAFLD (6,16).

Our study showed no significant differences in HRV indices between patients with AFLD and NAFLD. Similar findings were reported earlier. Assessment of autonomic neuropathy was done in 60 patients with well-preserved hepatic function; autonomic dysfunction was equally seen in patients with alcohol-related (47%) and non-alcohol related (44%) liver disease (8). Similarly, study on 64 patients with biopsy-proven liver disease (22 with alcoholic liver disease and 42 with non-alcoholic liver disease) and 29 age-matched controls also detected parasympathetic neuropathy in 45% of patients with alcoholic liver disease and 43% with non-alcoholic liver disease, which indicated that autonomic dysfunction was present in comparable frequency between the patients with alcoholic and non-alcoholic liver disease, suggesting that neurological defect might be a result of disturbed liver function (9).

We also observed a significant inverse relation between the HRV indices (LF, HF, SDRR) and serum GGT level in patients with ALD. This finding showed that higher the level of serum GGT lower the values of HRV indices, which may suggest that the more severe the liver damage, greater is the impairment of autonomic function. Earlier studies have shown increased GGT as strong predictor of fatty liver (17) and related with incident cardiovascular events, independent of alcoholic intake (12).

Negru *et al.,* (5) evaluated the correlations between HRV parameters and liver cirrhosis severity and found that the severity of liver disease was associated with greater impairment of HRV. Amaral *et al.,* (18) conducted a systematic review on cardiac autonomic modulation using HRV in non-alcoholic cirrhosis and observed an inverse association between HRV and the stages of liver cirrhosis. However, study by Gonzalez-Reimers *et al.,* (19) on 33 patients (20 cirrhotics and 13 non-cirrhotics) found a weak correlation between liver function impairment and both autonomic and peripheral neuropathy. Similarly, Thuluvath and Triger (17) reported that there was no strong correlation between severity of liver damage and the autonomic dysfunction in patients with well-

preserved liver function. Our study also observed no significant correlation between the HRV indices and liver enzymes (ALT, AST, GGT) in patients with NAFLD.

The exact mechanism underlying the association between fatty liver disease and autonomic dysfunction is not known. However, previous study had reported that plasma concentrations of angiotensin II are raised in patients with CLD and correlate with disease severity (20). It has been shown that infusion of angiotensin II causes a decrease in HRV and reduction in vagal discharge to heart which can be improved with the administration of captopril (21). Also, study by Matsuzaka *et al.,* (22) showed that there is association of fatty liver with insulin resistance. In another study Houghton *et al.,* (23) has also observed that insulin resistance was significantly associated with reduced heart rate variability indicating increased sympathetic and decreased parasympathetic activity.

Ziegler *et al.,* (24) found strong associations of both lower cardiovagal tone and baroreceptor sensitivity (BRS) with increased hepatic fat content. They also reported inverse association between liver fat content and several vagus modulated HRV indices as well as lower BRS. The hepatoportal vagal sensing of lipids may play a role in the pathophysiology of metabolic abnormalities such as hepatic insulin resistance apart from reflex regulation of feeding behavior (25). Elevated levels of free fatty acids in the portal vein decrease insulin clearance by the liver and those who display a better ability to clear fat from the liver would be at lower risk of developing hepatic complication. Therefore, preserved vagal activity could be protective in the context of hepatic fat accumulation.

The vagus nerve plays an important role in the regulation of metabolic homeostasis and cholinergic signaling, which is efferent vagus nerve mediated, controls immune function and pro-inflammatory response via the inflammatory reflex. It has been indicated that a complex interplay between subclinical inflammation (26), insulin resistance (27) and hepatic steatosis, driven by incipient inflammatory changes makes a substantial contribution to the early development of parasympathetic cardiac autonomic neuropathy (CAN). Therefore, it could be deduced from the above studies that angiotensin II, subclinical inflammation, and insulin resistance may contribute to autonomic disturbances in individuals with excessive liver fat.

CONCLUSION

Fatty liver disease is associated with significantly reduced HRV. No significant difference in HRV indices has been observed between AFLD and NAFLD. The greater the liver disease severity, the greater the HRV impairment has been shown by inverse relation between GGT and HRV indices. Reduced HRV and increased serum GGT have been documented to be associated with cardiovascular events and poor prognosis. Hence, assessment of autonomic function should be given due importance in the management of fatty liver disease with a view to help in improving the prognosis.

ACKNOWLEDGEMENTS

The study would never have been possible without the subjects who gave their consent for participation in the study. We are also thankful to technicians Mr. Chaoba and Ms. Zimick who helped us to carry out all the procedures.

CONFLICT OF INTEREST

There are no conflicts of interest about the research article.

REFERENCES

1. Mokdad, A.A., Lopez, A.D., Shahraz, S., Lozano, R., Mokdad, A.H., Stanaway, J., *et al.,* Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. BMC Med. 2014;12:145
2. Mathurin, P., Hadengue, A., Bataller, R., Addolorato, G., Burra, P., Burt, A., *et al.,* European association for the study of Liver EASL clinical practical guidelines management of alcoholic liver disease. J Hepatol 2012;57(2):399-420.
3. Kim, W.R., Lake, J.R., Smith, J.M., Skeans, M.A., Schladt, D.P., Edwards, E.B., *et al.,* OPTN/SRTR 2015 Annual Data Report: Liver. Am J Transplant 2017;17 Suppl 1:174-251.
4. Lunzer, M.R., Manghani, K.K., Newman, S.P., Sherlock, S., Bernard, A.G., Ginsburg, J. Impaired cardiovascular responsiveness in liver disease. Lancet 1975; ii: 382-385.
5. Negru, R.D., Cojocaru, D.C., Trifan, A., Dima-Cozma, C. Heart rate variability in patients with liver cirrhosis – a marker for autonomic disfunction. International Journal of Medical Dentistry. 2014:4(2).
6. Liu, Y.C., Hung, C.S., Wu, Y.W., Lee, Y.C., Lin, Y.H., Lin, C., *et al.,* Influence of Non-Alcoholic Fatty Liver Disease on Autonomic Changes Evaluated by the Time Domain, Frequency Domain, and Symbolic Dynamics of Heart Rate Variability. PLoS ONE 2013;8(4): e61803
7. Fleckenstien, J.F., Frank, S., Thuluvath, P.Z. Presence of autonomic neuropathy is a poor indicator in patients with advanced liver disease. Hepatol 1996; 23:471-475.
8. Hendrickse, M.T., Triger, D.R. Peripheral and cardio-vascular autonomic impairment in chronic liver disease: prevalence and relation to hepatic function. J Hepatol 1992; 16:177-183.
9. Thuluvath, P.J., Triger, D.R. Autonomic neuropathy in chronic liver disease. J Med 1989;72: 737-747.
10. Anderson, K.M. Cholesterol, and mortality. 30 years of follow-up from the Framingham study. JAMA 1987; 257:2176-2180.
11. Lee, H., Shin, N.W., Lee, T.H., Yang, H.K., Ahn, E., Yoon, J.M., *et al.,* Association between change in serum aminotransferase and mortality. Medicine 2016; 95.
12. Fraser, A., Harris, R., Sattar, N., Ebrahim, S., Smith, G.D., Lawlor, D. Gamma-glutamyltransferase is associated with incident vascular events independently of alcohol intake. Arter. Thromb. Vasc. Boil. 2007; 27: 2729-2735.
13. Coelho, L., Saraiva, S., Guimaräes, H., Freitas, D., Providência, L.A. Autonomic function in chronic liver disease assessed by heart rate variability study. Rev Port Cardiol 2001 Jan; 20(1):25-36.

14. Iga, A., Nomura, M., Sawada, Y., Ito, S., Nakaya, Y. Autonomic nervous dysfunction in patients with liver cirrhosis using 123I-metaiodobenzylguanidine myocardial scintigraphy and spectrum analysis of heart-rate variability. J Gastroenterol Hepatol. 2003;18: 651-659.
15. Milovanovic, B., Milinic, N., Trifunovic, D., Krotin, M., Filipovic, B., Bisenic, V., *et al.,* Autonomic dysfunction in alcoholic cirrhosis and its relation to sudden cardiac death risk predictors. Gen Physiol Biophys 2009; 28: 251-261.
16. Kumar, M.S., Singh, A., Jaryal, A.K., Ranjan, P., Deepak, K.K., Sharma, S., *et al.,* Cardiovascular autonomic dysfunction in patients of nonalcoholic fatty liver disease. Internat J Hepatol 2016. 1-8.
17. Choi, K.M., Han, K., Park, S., Chung, H.S., Kim, N.H., Yoo, H.J., *et al.,* Implication of liver enzymes on incident cardiovascular diseases and mortality: A nationwide population-based cohort study. Sci Rep 2018; 8: 3764.
18. Amaral, J.A.T.D., Salatini, R., Arab, C., Abreu, L.C., Valenti, V.E., Monteiro, C.B.M., *et al.*, Non-Alcoholic Cirrhosis and Heart Rate Variability: A Systematic Mini-Review. Medicina 2020; 56(116)
19. Gonzalez-Reimers, E., Alonso-Socas, M., Santolaria-Farnandez, F., Hernandez-Pena, J., Conde-Martel, A., Rodriguez-Moreno, F. Autonomic and peripheral neuropathy in chronic alcoholic liver disease. Drug Alcohol Depend 1991;27: 219-222.
20. Arroyo, V., Planas, R., Gaya, J., Deulofeu, R., Rimola, A., Perez-Ayuso, R.M., *et al.*, Sympathetic nervous activity, renin-angiotensin system and renal excretion of prostaglandin E2 In cirrhosis. Relationship to functional renal failure and sodium and water excretion. Eur J Clin Invest 1983;13(3):271-278.
21. Dillon, J.F., Nolan, J., Thomas, H., Williams, B.C., Neilson, J.M., Bouchier, I.A., *et al.,* The correction of autonomic dysfunction in cirrhosis by captopril. J. Hepatol 1997; 26:331-335.
22. Matsuzaka, T., Shimano, H. Molecular mechanisms involved in hepatic steatosis and insulin resistance. J Diabetes Investig 2011;2(3):170-175.
23. Houghton, D., Zalewski, P., Hallsworth, K., Cassidy, S., Thoma, C., Avery, L., *et al.,* The degree of hepatic steatosis associates with impaired cardiac and autonomic function. J Hepatol 2019; 70:1203-1213.
24. Ziegler, D., Strom, A., Kupriyanova, Y., Bierwagen, A., Bonhof, G.J., Bodis, K., *et al.,* Association of Lower Cardiovagal Tone and Baroreflex Sensitivity with Higher Liver Fat Content Early in Type 2 Diabetes. J Clin Endocrinol Metab 2018; 103(3): 1130-1138.
25. Yi, C.X., la Fleur, S.E., Fliers, E., Kalsbeek, A. The role of the autonomic nervous liver innervation in the control of energy metabolism. Biochim Biophys Acta. 2010; 1802:416-431.
26. Herder, C., Schamarek, I., Nowotny, B., Carstensen-Kirberg, M., Straßburger, K., Nowotny, P., *et al*., Inflammatory markers are associated with cardiac autonomic dysfunction in recent-onset type 2 diabetes. Heart 2016;103(1):63-70.
27. Ziegler, D., Strom, A., Bonhof, G., Puttgen, S., Bodis, K., Burkart, V., *et al.,* Differential associations of lower cardiac vagal tone with insulin resistance and insulin secretion in recently diagnosed type 1 and type 2 diabetes [published online ahead of print November 4, 2017]. Metabolism. doi: 10.1016/j.metabol.2017.10.013.