Original Research Article

Correlation of N-terminal pro-brain natriuretic peptide levels in non-alcoholic fatty liver disease

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Abstract

Background: Many organ systems are found to be affected in NAFLD including heart.NT-pro BNP determination has been proven to be useful and accurate for ruling out the diagnosis of heart dysfunction. **Aim:** To characterize the correlation between NT-proBNP and nonalcoholic fatty liver disease (NAFLD). **Method:** For this study, 60 cases of NAFLD were examined and evaluated in Department of Medicine, N.S.C.B. Medical College & Hospital, Jabalpur (M.P.) All study subjects were examined by same radiologist for detecting fatty liver and grading by ultrasonography of abdomen; and all patients undergo fibroscan by same technician for measuring liver stiffness measurement . Similarly, NT-proBNP levels were also measured using a commercially available immunochemical system. **Result:** Our study shows that NT-pro BNP levels are significantly lower in patients of NAFLD without diastolic dysfunction(mean±SD46.17±18.74) and compared to NAFLD patients with diastolic dysfunction(160.89±42.11). **Conclusion:** Difference in NT- pro BNP levels was significantly observed in NAFLD patients with or without diastolic dysfunction. Therefore, NT ProBNP can be used as a diagnostic tool for diagnosis of NAFLD in patients without diastolic dysfunction.

Keywords: diastolic dysfunction, NAFLD, NASH, NT-proBNP

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Introduction

Non Alcoholic Fatty Liver Disease (NAFLD) is deposition of extra fat in the liver of people who does not consume alcohol. It is a common disorder that does not affect in its early stages, but it can lead to major liver damage, including cirrhosis. Normally, liver contains some fat, but if liver contains more than 5% – 10% percent of the weight then it is called a fatty liver (steatosis)[1]. People with obesity, type 2 diabetes, high cholesterol, hypothyroidism, hypopituitarism, sleep apnoea, PCOS (Polycystic ovary syndrome), high triglyceride levels, use of corticosteroids are at higher risk of NAFLD[2]. It can affect people of different ages, including children and adults.

NAFLD is classified into two types based on severity: simple fatty liver and NASH (nonalcoholic steatohepatitis). In simple fatty liver disease, there is fat in liver but little or no inflammation or liver cell damage. It typically does not progress to cause liver damage or complications. On the other hand, patients with NASH have hepatitis, inflammation and liver cell damage in addition to fat in liver. Inflammation and liver cell damage can cause fibrosis or scarring of the live therefore, NASH may result in liver cirrhosis and sometimes in hepatocellular carcinoma[3].

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NAFLD not only affects liver but it is a multisystem disease that affects many organ systems including heart. It is reported that NAFLD cause early changes in cardiac substrate metabolism, producing functional, structural and arrhythmic consequences that are potentially linked to an increased risk of new-onset heart failure. In response to increased wall tension, heart primarily produce and release natriuretic peptides in the circulation. Circulating concentrations of several cardiac natriuretic peptides were raised including NT-proBNP (N-terminal pro-brain natriuretic peptide). They can be raised in both symptomatic and asymptomatic patients with left ventricular dysfunction[4].

Natriuretic peptides are released in response to an increased cardiac volume and pressure overload[5]. These peptides are primarily synthesized as high molecular weight precursors; later, they are cleaved into an amino-terminal segment of the precursor molecule(NT-proBNP) and the biologically active peptides[6]. Studies show appearance of NT-pro BNP in response to wall stress as a sensitive biomarker for the diagnosis, prognosis and treatment of heart failure[7,8].

In cell, natriuretic peptides binds to its receptors -A and -B. Binding of NP to its receptors leads to activation of the gene that codes for the peroxisomeproliferator-activated receptor γ coactivator-1 α (PGC1A)[9] and stimulates an increase in mitochondrial density, oxygen consumption and an increase in insulin sensitivity[10,11] and increased lipolysis in adipose tissue independently from catecholamine induced lipolysis[12,13]. These metabolic actions may

provide a biological explanation for the accumulation of fat and the development of NAFLD associated with low levels of NT-proBNP. Currently, the association between plasma NT-proBNP levels and the histological severity of NAFLD were not reported. Therefore, the current study is conducted to find the correlation and association between NT-proBNP and liver fat.

Methodology

Study Subjects

The present study was carried out in N.S.C.B. Medical College Hospital, Jabalpur. (M.P.) Participants of at least 18 years of age with known or suspected Non alcoholic fatty liver disease (NAFLD) including different spectrum of NAFLD patients [nonalcoholic fatty liver (NAFL), Nonalcoholic steatohepatitis (NASH) and NASH-related cirrhosis were included in the study.The study was approved by institutional review boards and written informed consent was obtained from every participant prior to data collection.

Measurementof Body Mass Index and Blood Pressure

It was doneusing standard protocol.[14,15]

Measurementof Fasting Lipid Profile and Blood sugar

It is measured by using RandoxModerna machine.

Measurementof Liver Fat

NAFLD was detected by means of ultrasonography done by single experienced radiologist, using a B-mode ultrasonography of frequency of 3–5 MHz .An increase in hepatic echogenicity was noted .The enhancement and differential loss in the periportal intensity and the vascular wall due to increased hyperechogenicity in the liver parenchyma is also noted.

Echocardiography

The E/A ratio is the ratio of the early (E) to late (A) ventricular filling velocities. In a healthy heart, the E velocity is greater than the A velocity. The reversal of the E/A ratio ('A' velocity becomes greater than 'E' velocity) is often accepted as a clinical marker of diastolic dysfunction, in which the left ventricular wall becomes so stiff as to impair proper filling, which can lead to diastolic heart failure.[16]

Transient elastography

It was done using FIBROSCAN 402 ® device; To avoid any misinterpretation, it was done after 8hours fasting in all subjects around 10 AM. When ultrasound was used, an ultrasoundprobe emits a vibration that creates a shear wave within the liver. This shear wave corresponds to liver stiffness. In addition, liver stiffness was significantly higher in patients with stage 3 or 4 fibrosis than lower stages [17,18]. NAFLD spectrum on the basis of LSM[liver stiffness measurement] on FibroScan® :<7 kPa- NAFL, ≥7 to <13- NASH, ≥13- NASH related cirrhosis.

NT-proBNP level measurement

Peripheral venous blood was collected in a plain tube under aseptic precautions.To avoid the diurnal variations of NT-pro BNP, all the samples were collected at the same time(between 9 am to 10am). NT-pro BNP levels were measured by Immunoflourescence technique using Roche-Cobas e411 analyser.

Statistical Analysis

The collected data was fed into an excel spreadsheet and then tabulated. Data was statistically analysed using t-test, chi-square test and Karl Pearson's correlation using SPSS version 20 and Microsoft excel and $p<0.05$ was considered to be statistically significant.

Results

A total of 60 patients of NAFLD in N.S.C.B. Medical College &Hospital , Jabalpur (M.P.) from March 2019 to Aug 2020 who were willing to take part in the study and gave a written informed consent and who fulfilled the inclusion and exclusion criteria. In our study, out of 60 patients diagnosed as NAFLD, 39 (65%) were classified as NAFL, 17 (28.3%) as NASH and 4(06.6%) as NASH related cirrhosis.

- **Diastolic Dysfunction**

In our study, 40(66%) patients had diastolic dysfunction. 58.97% of NAFL patients,82% of NASH patients; 75% of NASH related Cirrhosis patients had diastolic dysfunction.

- **Anthropological analysis**

Table 1 shows the mean value along with standard deviation of different clinical and biochemical parameters of NAFLD. In our study 53.33% of patients of NAFLD had increased waist circumference (≥90 in men & ≥80cms in women) as defined by criteria established for Asian Indians. In our study mean waist circumference in NAFLD cases 88.87±06.63. The mean BMI was 27.81±3.79 kg/m2. According to BMI 6% of patient were overweight,(BMI 23-24.99), 35% were pre obese and 14% were obese (BMI >30) according to Indian Asian criteria of BMI. The mean BMI within the NAFLD group was found as NAFL: 27.7±3.8 , NASH : 28.1±4.3 , NASH related cirrhosis : 27.8±0.3respectively.

- **Laboratory Analysis**

Fasting blood sugars is a contradictory parameter of NAFLD which we found to be 112.09±48.16 . The mean total cholesterol was 194.65+56.42 mg/dl and hypercholesterolemia (>200mg/dl) was found in 40% patients. 21.66% of patients had hypertension (≥130/85 mmHg) as per the NCEP ATP III criteria.

Based on serum Triglyceride level, hypertriglyceridemia (>150mg/dl) was present in 58.33% cases with a mean of 175.59±84.33mg/dl. In our study mean HDL cholesterol was 42.60± 13mg/dl and low level of HDL Cholesterol (<40mg/dl in male,<50mg/dl in female) was present in 75% of patients.

- **Echocardiographic Analysis**

Increased thickness of interventricular septum which is directly correlated to LV mass is found in NAFLD group; In this study, increased interventricular thickness was seen in 46(76.66%) patients(p value- 0.043). The mean IVS was 1.19± 0.22 in our study.

E/A ratio less than 1 is suggestive of diastolic dysfunction; In this study, 23.1% of NAFL patients, 58.8% of NASH patients and 50% of NASH related cirrhosis patients has E/A ratio less than 1 which shows that with progression of NAFLD spectrum of disease , diastolic dysfunction (E/A <1) prevalence increases(p value 0.029). The mean E/A ratio in our study is 1.22 ± 0.43.

Table 1: Clinical And Biochemical Parameters Of NAFLD Patients.

Variables	N	Minimum	Maximum	Mean ± SD
AGE(years)	60	20.00	67.00	44.87±11.68
HEIGHT(metres)	60	1.48	1.65	1.56±0.04
WEIGHT(kilogram)	60	50.00	88.00	67.90±7.72
BMI(kg/m^2	60	19.50	38.00	27.81±3.79
WAIST (cm)	60	68.00	102.00	88.67±6.63
SBP(mm hg)	60	90.00	160.00	122.08±14.16
DBP(mm hg)	60	60.00	110.00	79.30±9.57
IVS THICKNESS(cm)	60	0.90	2.30	1.19±0.22
E/A RATIO	60	0.57	2.00	1.22±0.43
TOTAL CHOLESTEROL(mg/dl)	60	116.00	485.00	194.65±56.42

TRIGLYCERIDE(mg/dl)	60	30.70	465.00	175.59±84.33
HDL(mg/dl)	60	21.00	98.60	42.60±13.00
AST(IU/L)	60	14.00	97.50	36.46±17.32
ALT(IU/L)	60	10.00	186.80	40.72±30.91
FBS(mg/dl)	60	61.80	296.00	112.09±48.16
LSM(kpa)	60	2.60	69.10	8.09±8.69

- **NT-pro BNP**

The NT ProBNP levels were measured for all 60 NAFLD patients. Table 2 shows the Correlation of NT-pro BNP Levels With Diastolic Dysfunction. It is observed that patients who had NT-pro BNP ≤125 and >125 have 34.48% and 96.77% diastolic dysfunction respectively. The analysis revealed that >125 NT-PRO BNP was the significant risk factor of diastolic dysfunction as compared with ≤125 NT-PRO BNP (Chi square = 26.16; P<0.0001).

Table 2: Correlation OfNt Pro Bnp Level With Diastolic Dysfunction

NT-PRO BNP(pg/ml)	Diastolic dysfunction present	Diastolic dysfunction absent	Chi square and p value
≤125	10 (34.48%)	19 (65.52%)	26.16; P<0.0001
>125	30(96.77%)	1 (3.23%)	

In our study, out of 60 patients who had undergone NT-proBNP levels, 40(66.66%) patients have diastolic dysfunction; out of them 30(75%) patients have NT-proBNP levels in the higher range (>125pg/ml) and out of 20 (33.33%) patients who don't have diastolic function,19(96.67%)

Table 3: Correlation Of NAFLD Spectrum Of Disease With Nt-Pro Bnp Levels With And Without Diastolic Function

SPECTRUM	Diastolic dysfunction	NT-pro BNP (pg/ml) Number (percentage)		Mean NT-pro BNP	Total	P value
		≤125	>125			
NAFL	NO	16 (100)	0 (0)	41.3	16	<0.0001
	YES	7 (30.43)	16 (69.57)	138.3	23	
Total		23 (58.97)	16 (41.03)		39	
NASH	NO	3 (100)	0 (0)	54.7	3	0.029
	YES	3 (21.43)	11 (78.57)	164.9	14	
Total		6 (35.29)	11 (64.71)		17	
NASH related Cirrhosis	NO	0 (0)	1 (100)	212	1	-
	YES	0 (0)	3 (100)	208	3	
Total		0 (0)	4 (100)		4	

Discussion

The study aimed to evaluate the correlation between NT- pro BNP levels in NAFLD patients with or without diastolic dysfunction.

Table 4 shows various previous studies correlating NT-pro BNP levels with NAFLD. This study was in accordance with the previous studies in patients with NAFLD, illustrating NT-pro BNP levels were in lower quantile for NAFLD patients. Our study shows lower levels of NT-pro BNP levels in NAFLD patients without any diastolic dysfunction. In our study, NT-pro BNP levels were lower in NAFLD patients as compared to NASH patients.

Table 4: Various previous studies correlating NT-pro BNP levels with NAFLD

Study, journal and author	*Variable*	*Sample size*	*Result*
Journal: METABOLISM Year:2016 Author: Otto A Sanchez et al[19]	NT-pro BNP and NAFLD	4529	RP for NAFLD decreased by 30% from the lowest to the highest quintile of NT-proBNP, p = 0.01.
Journal: Nutrition, Metabolism and Cardiovascular Diseases Year: 2020 Author:Zeng-Pie Qiao et al[20]	NT-pro BNP and NAFLD	351	After stratification of patients by plasma NT-proBNPtertiles; compared to those in the 1st tertile (NT-proBNP ≤16 pg/ml), the odds ratio for NASH was 0.52 (95% CI 0.29–0.95) in patients in the 2nd tertile (NT-proBNP of 17–33 pg/ml) and 0.49 (95% CI 0.26–0.93) in those in the 3rd tertile (NT-proBNP ≥34 pg/ml) of plasma NT-proBNP levels.
Journal: Diabetes & Metabolism Year:2019 Author: Marie Louise Johansen et al[21]	NT-pro BNP and NAFLD in Diabetes mellitus	120	NAFLD was found in 57 (48%) of the T2D patients, who also had significantly lower NT-proBNP (P = 0.002) levels compared with patients without NAFLD. The odds ratio for the presence of NAFLD was increased by 2.9 (P = 0.048) for NT-proBNP levels < 45 ng/L.

The mean NT PRO BNP among patients with and without diastolic dysfunction was 160.89±42.11 and 46.17±18.74 respectively. Further analysis of mean difference was found statistically significant (t = 8.83; P<0.0001).

Thus NT-pro BNP levels can be used as a biomarker for diagnosis of NAFLD patients who have normal echocardiographic indices (intraventricularseptal thickness, E/A ratio) and can be used to initiate timely lifestyle modification to prevent diastolic dysfunction in patients of NAFLD.

NT-pro BNP can also be used as a biomarker for diagnosis of isolated diastolic dysfunction in NAFLD patients as all patients with diastolic dysfunction have NT-pro BNP >125mg/dl.

There are certain limitations in our study. We did not compare the NT-pro BNP levels of NAFLD patients with healthy population in our study.

Conclusion

Our study shows that NT-pro BNP levels are significantly lower in patients of NAFLD without diastolic dysfunction (mean±SD46.17±18.74) compared to NAFLD patients with diastolic dysfunction(160.89±42.11). So, NT- pro BNP can be used as a diagnostic tool for diagnosis of NAFLD in patients without diastolic dysfunction.

References

1. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018: 67(1); 328-357
2. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat rev. Gastroenterol&hepatol 2018; 15(1): 11.
3. Arvaniti VA, Thomopoulos KC, Tsamandas A, Makri M, Psyrogiannis A, Vafiadis G, et al. Serum adiponectin levels in different types of non alcoholic liver disease. Correlation with steatosis, necroinflammation and fibrosis. Acta Gastro-EnterologicaBelgica 2008; 71(4): 355-360.
4. Hunt PJ, Richards AM, Nicholls MG, Yandle TG, Doughty RN, Espiner EA. Immunoreactive amino-terminal pro-brain natriuretic peptide (NT-PROBNP): a new marker of cardiac impairment. Clinical endocrinology. 1997 Sep;47(3):287-96.
5. . Kremastinos D T, Tsetsos G A, Tsiapras D P, Karavolias G K, Ladis V A, Kattamis C A. Heart failure in beta thalassemia: a 5-year follow-up study. *Am J Med.* 2001;111(05):349–354.
6. Shimizu H, Masuta K, AonoKetal.Molecular forms of human brain natriuretic peptide in plasma *ClinChimActa* 2002316(1-2):129–135.
7. Daniels L B, Maisel A S. Natriuretic peptides. *J Am CollCardiol*. 2007;50(25):2357–2368.
8. vanKimmenade R R, Januzzi J L., Jr Emerging biomarkers in heart failure. *Clin Chem.* 2012;58(01):127–138.
9. Engeli S, Birkenfeld AL, Badin PM, Bourlier V, Louche K, Viguerie N, et al. Natriuretic peptides enhance the oxidative capacity of human skeletal muscle. *Journal of Clinical Investigation.* 2012;122:4675–9.
10. Miyashita K, Itoh H, Tsujimoto H, Tamura N, Fukunaga Y, Sone M, et al. Natriuretic peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle mitochondrial biogenesis and prevent obesity. *Diabetes.* 2009;58:2880–92.
11. Souza SC, Chau MD, Yang Q, Gauthier MS, Clairmont KB, Wu Z, et al. Atrialnatriuretic peptide regulates lipid mobilization and oxygen consumption in human adipocytes by activating AMPK. *BiochemBiophys Res Commun.* 2011;410:398–403.
12. Lafontan M, Moro C, Berlan M, Crampes F, Sengenes C, Galitzky J. Control of lipolysis by natriuretic peptides and cyclic GMP. *Trends in endocrinology and metabolism: TEM*. 2008;19:130–7.
13. Sengenes C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic peptides: a new lipolytic pathway in human adipocytes. *FASEB Journal.* 2000;14:1345–51.
14. Greenwood J, Narus S, Leiser J, Egger M. Measuring Body Mass Index According to Protocol: How are Height and Weight Obtained?. Journal for Healthcare Quality. 2011;33(3):28-36.
15. James G, Gerber L. Measuring arterial blood pressure in humans: Auscultatory and automatic measurement techniques for human biological field studies. American Journal of Human Biology. 2017;30(1):e23063.
16. Leoni S, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. World journal of gastroenterology 2018; 24(30): 3361.
17. Takeda, J., Yasuda, T., Kimura, M., Nakaya, M., Fujii, H., Nakayama, Y., ...& Seki, S. (2006). Noninvasive diagnosis of nonalcoholic steatohepatitis using elastometry. Hepatology, 44(Suppl 1), 652A.
18. Mikolasevic, I., Milic, S., Orlic, L., Stimac, D., Franjic, N., &Targher, G. (2016). Factors associated with significant liver steatosis and fibrosis as assessed by transient elastography in patients with one or more components of the metabolic syndrome. Journal of Diabetes and its Complications, 30(7), 1347-1353.
19. Sanchez, O., Lazo-Elizondo, M., Zeb, I., Tracy, R., Bradley, R., &Duprez, D. et al. (2016). Computerized tomography measured liver fat is associated with low levels of N-terminal pro-brain natriuretic protein (NT-proBNP). Multi-Ethnic Study of Atherosclerosis. *Metabolism*, *65*(5), 728-735.
20. Qiao, Z., Zheng, K., Zhu, P., Gao, F., Ma, H., & Li, G. et al. (2020). Lower levels of plasma NT-proBNP are associated with higher prevalence of NASH in patients with biopsy-proven NAFLD. *Nutrition, Metabolism And Cardiovascular Diseases*, *30*(10), 1820-1825.
21. Johansen, M., Schou, M., Rasmussen, J., Rossignol, P., Holm, M., &Chabanova, E. et al. (2019). Low N-terminal pro-brain natriuretic peptide levels are associated with non-alcoholic fatty liver disease in patients with type 2 diabetes. *Diabetes & Metabolism*, *45*(5), 429-435. doi: 10.1016/j.diabet.2018.11.003.

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