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Estimation of Serum Ethanol in Patients with Non-alcoholic Fatty Liver Disease (NAFLD)

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KEYWORDS

Cirrhosis, ethanol, fibrosis, inflammatory, NAFLD

ABSTRACT:

Background: Being a common multifactorial disease, Nonalcoholic fatty liver disease (NAFLD) is poorly understood liver disease whose incidence is globally rising. Increased level of endogenous ethanol is associated with increased oxidative stress which directly leading to activation of inflammatory cascade, ultimately causes fatty liver and cirrhosis.

Objective: To evaluate the relationship of serum ethanol level with different stages of NAFLD patients and healthy individuals.

Methods: This observational study was occurred in the Department of Hepatology, BSMMU from January 2022 to August 2022. All participants who attended outpatient Department of Hepatology, BSMMU with ultrasonographic evidence of Fatty Liver, who fitted the inclusion and exclusion criteria, and gave informed written consent for liver biopsy were assigned and liver biopsy was done.

Results: This current observational study revealed that 11 patients were NASH, 29 patients were non-NASH and 40 healthy individuals. It is evident that, majority of the study participants were male (55.0%). the healthy individual group and NAFLD group of participants were significantly different in term of Total cell count (--x109/L), Neutrophil, lymphocyte, Fasting blood sugar (mmol/l), blood sugar 2 hours after breakfast (mmol/L), HbA1c (%), HDL (mg/dl), LDL (mg/dl), ALT (U/L), ALT (U/L) (p value: <0.05). among the patients with NAFLD, 27.5% were suffering from NASH, whereas, 72.5% were suffering from non-NASH NAFLD. Moreover, patients with Serum Ethanol level of ≥1.50 (mg/dl) were significantly 7.877 times at risk (95% CI: 1.49-41.383) of developing NASH, and patients with Serum triglyceride level of ≥150 mg/dl were non significantly 2.625 times at risk (95% CI: 0.400-17.224) of developing NASH.

Conclusion: Serum ethanol level was higher in NASH patients in comparison to non-NASH patients and healthy individuals. Also, significant relation comparing with NAS score and serum ethanol level was observed.

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Introduction:

The alarming growth of Nonalcoholic fatty liver disease (NAFLD) is a global public health concern with its increasing prevalence in the presence or absence of metabolic comorbidities. Non-alcoholic fatty liver disease (NAFLD) is currently the most common cause of chronic liver disease in both children and adults worldwide, with an estimated global prevalence of 25.2% (range,22.1–28.6%) [1]. This liver disease is devoid of causes for secondary hepatic fat accumulation such as significant alcohol consumption, the use of steatogenic medication, or hereditary disorders [2]. NAFLD, a continuum of liver abnormalities from non-alcoholic fatty liver to non-alcoholic steatohepatitis, has a variable course, but it can lead to cirrhosis and liver cancer; thus, the early pathology of NAFLD is important [3]. NAFLD includes 90% of cases who have at least one component of the metabolic syndrome and as many as 33% have three or more components, NAFLD is recognized as the hepatic manifestation of the metabolic syndrome [4].

The undiscovered mechanisms related to NAFLD development and progression has been gone under consideration. Although there is increasing evidence that NAFLD is associated with dyslipidemia, obesity, metabolic and insulin resistance syndrome and changes in intestinal flora, the precise pathogenesis underlying the condition remains unclear. Recent studies illustrated that the gut–liver axis is a key player in the pathogenesis of NAFLD because the introduction of bacterial derivatives into the portal vein circulation may trigger innate immunity and then lead to liver inflammation [5,6,7,8].

Recently, the gut-liver axis has been proposed as a key player in the pathogenesis of NAFLD, as the passage of bacteria-derived products into the portal circulation could lead to a trigger of innate immunity, as a result, it may lead to liver inflammation [9].

In addition, in patients with liver injury, the prevalence of intestinal disorders is elevated endogenous ethanol production is increased, and the prevalence of intestinal permeability and bacterial translocation is higher. In the 1850s, scientists discovered that small amounts of ethanol are produced naturally in the human body [10].

In recent years, changes in the gastrointestinal microbiota and increased endogenous gastrointestinal ethanol synthesis have been considered critical in the development of NAFLD. Endogenous ethanol is originated mainly from the microbiota in the gastrointestinal tract. The generation of endogenous ethanol is related to the microorganisms in the gastrointestinal tract. Being a major enzyme, alcohol dehydrogenase (ADH) is considered as controlling ethanol synthesis in the 2, 3-butanediol pathway.

Methods:

This observational study in the department of Hepatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbagh, Dhaka started enrolling from January, 2022 to August, 2022. The study population was patients having nonalcoholic fatty liver disease (NAFLD) and healthy individuals attending the outpatient department of Hepatology, BSMMU. After enrollment, the study subjects were purposively divided into the following three groups -

NASH Group: NAFLD patients, aged 18 – 60 years who had NAS score ≥5 up to 8, confirmed by liver biopsy.

Non-NASH Group: NAFLD patients age within 18 – 60 years who had NAS score

<5, confirmed by liver biopsy.

Healthy Group: Ultrasonographic evidence of normal liver parenchyma, aged within 18 to 60 years. 40 NAFLD patients and 40 healthy individuals were included in this study considering the inclusion and exclusion criteria.

After receiving histopathology report, the patients were grouped into NASH and non- NASH categories. Those with NAFLD activity score (NAS) between 5 to 8 were considered as NASH and those with NAS 0 to 4 were considered non-NASH fatty liver (NNFL). Total 40 patients with NAFLD were biopsied.

Inclusion criteria

For NAFLD patients:

- Patients with ultrasonographic evidence of fatty liver.

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- Age: 18 to 60 years.

For healthy individuals:

- Ultrasonographic evidence of normal liver parenchyma
- Age: 18 to 60 years.

Exclusion criteria

Patients with history of alcohol intake.

- Patients with history of taking drugs that may cause fatty liver (i.e.

Tamoxifen, Valproic Acid, Amiodarone, Methotrexate).

- Chronic liver disease due to any cause (HBV, HCV, Wilson's disease, Haemochromatosis, Drug induced liver injury etc.) and hepatocellular carcinoma.
- History of taking drugs that may cause fatty liver (i.e., Tamoxifen, Valproate, Amiodarone, MTX, Corticosteroid)
- Pregnancy
- Patient with co-morbid conditions (COPD, CKD, CCF etc.)

Data collection was conducted in the Department of Hepatology, BSMMU. Patients were included after primary screening with inclusion criteria. Structured questionnaire was used to collect all necessary information and record all the required data that was used for analysis. Informed written consent was taken from each participant before collecting data.

Quantitative data were presented as mean ± SD & qualitative data were presented in percentage. All data were analyzed by SPSS (version 22.0, IBM Corp: Armonk, NY, USA). Qualitative data were analyzed by Chi-square test & quantitative data were analyzed by student's t-test and ANOVA test. Kruskal Wallis test and Mann Whitney U test were used to compare laboratory parameters & measurements obtained in case and control group. Receiver operator characteristics (ROC) analysis was done of serum ethanol level to predict NASH. Binary logistic regression analysis was done to find out best predictor. A statistically significant result was considered when p value less than 0.05.

Result:

Table 1: Socio-demographic characteristics of the study population (N=80)

Table 1 resembles socio-demographic characteristics of the study population. Table I is showing that majority of the healthy individual (35.0%) and NAFLD (42.5%) were from 31-40 years age group. The mean ± SD age of the healthy individual was 39.48± 11.40 years, whereas the mean ± SD age of the NAFLD patients was 39.55±9.14 years and in both group majorities of the patients were male.

Demographic characteristics		**Healthy individuals n1=40**	**Non-alcoholic fatty liver disease n2=40**	**Total**	**P value**
Age	18-30 years	11 (27.5)	7 (17.5)	18 (22.5)	0.165^{NS}
	31-40 years	14 (35.0)	17 (42.5)	31 (38.8)	
	41-50 years	5 (12.5)	11 (27.5)	16 (20.0)	
	51- 60 years	10 (25.0)	5 (12.5)	15 (18.8)	
	Mean ± SD	39.48± 11.40	39.55±9.14	39.51±10.27	$^{a}0.974^{NS}$
	Median (range)	35 (22-60)	39 (23-59)	37 (22-60)	

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Gender	Male	23 (57.5)	21 (52.5)	44 (55.0)	0.653^{NS}
	Female	17 (42.5)	19 (47.5)	36 (45.0)	

Figure 1: Distribution of the participants according to gender (N=80)

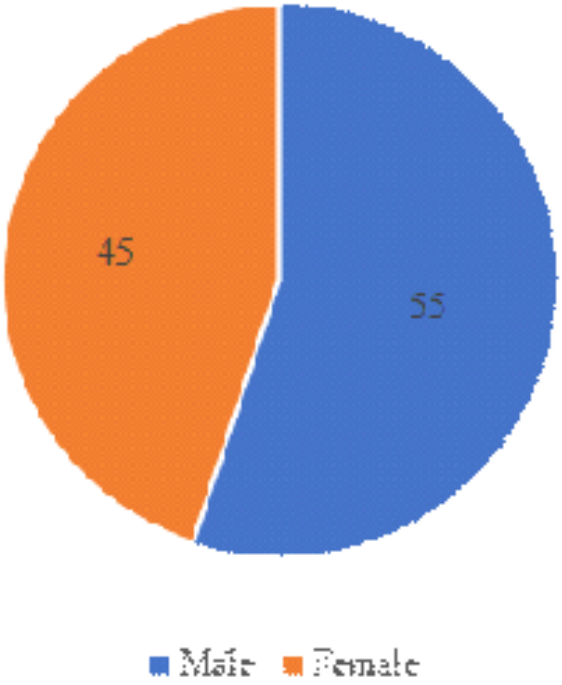

Figure 1 resembles distribution of the participants according to gender. It is evident that, majority of the study participants were male (55.0%).

Table 2: Baseline characteristics of the study population (N=80)

Table 2 is showing that the healthy individual group and NAFLD group of participants were significantly different in term of Total cell count (--x10^9/L), Neutrophil, lymphocyte, Fasting blood sugar (mmol/l), blood sugar 2 hours after breakfast (mmol/L), HbA1c (%), HDL (mg/dl), LDL (mg/dl), ALT (U/L), ALT (U/L) (p value: <0.05).

moBaseline characteristics		**Healthy individuals n1=40**	**Nonalcoholic fatty liver disease n2=40**	P value
	Mean ± SD	12.68±1.06	13.14±1.37	

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Hb (gm/dl)	Median (range)	12.70 (10.50 - 14.30)	13.10 (10.00-15.80)	[a]0.099[NS]
TC(--x10^9/L)	Mean ± SD	6.84±1.26	8.33±1.70	<0.001[S]
	Median (range)	6.70 (5.30-11.0)	8.00 (3.67-14.50)	
Neutrophil (%)	Mean ± SD	62±5	58±7	[a]0.024[S]
	Median (range)	62 (51-72)	59 (44-71)	
Lymphocyte (%)	Mean ± SD	29±5	34±7	0.002[S]
	Median (range)	30 (20-42)	34 (21-47)	
FBS (mmol/L)	Mean ± SD	5.16±0.91	5.96±1.90	0.041[S]
	Median (range)	5.20 (3.90-7.30)	5.40 (3.80-13.90)	
2HABF(mmol/L)	Mean ± SD	6.54±2.14	8.24±3.99	<0.001[S]
	Median (range)	5.90 (4.90-14.20)	6.80 (5.50-27.30)	
HbA1c (%)	Mean ± SD	5.40±0.85	6.01±1.44	0.038[S]
	Median (range)	5.35 (4.20-7.80)	5.50 (4.60-11.60)	
	Mean ± SD	2.15±0.76	1.82±.51	

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HOMA-IR	Median (range)	2.16 (1.13-6.10)	1.80 (0.90-3.70)	0.001[S]
TSH (µIu/ml)	Mean ± SD	1.92±0.57	4.27±7.07	0.080[NS]
	Median (range)	1.92 (0.99-3.20)	2.27 (0.27-34.66)	
Total cholesterol (mg/dl)	Mean ± SD	186.00±38.03	191.90 ±50.70	[a]0.558[NS]
	Median (range)	188.00 (119-290)	180.00 (101-322)	
LDL(mg/dl)	Mean ± SD	125.93±26.78	109.55±37.32	[a]0.027[S]
	Median (range)	125.00 (71-212)	104.40 (35-213)	
HDL(mg/dl)	Mean ± SD	45.98±7.39	39.30±10.27	[a]0.001[S]
	Median (range)	45.00 (32.00-63.00)	38.00 (22-67)	
	Median (range)	23.00 (13.0-35.0)	39.00 (12-138)	
ALT(U/L)	Mean ± SD	29.78±7.78	65.07±37.68	<0.001[S]
	Median (range)	31.00 (15.00-40.00)	58.50 (15-176)	

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Figure 2: Distribution of NAFLD by NAFLD Activity Score (NAS) (N=40)

Table 2 is showing that among the patients with NAFLD, 27.5% were suffering from NASH, whereas, 72.5% were suffering from non-NASH NAFLD.

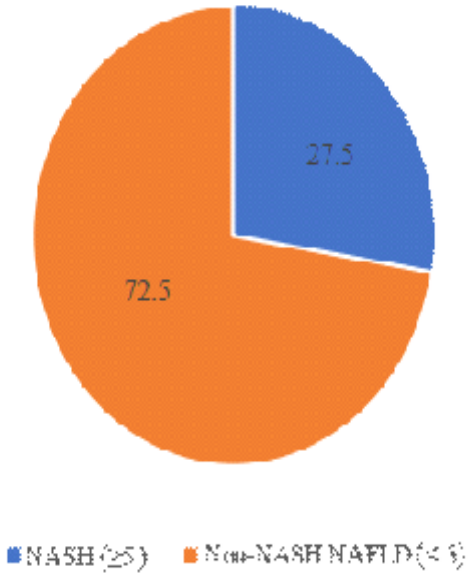

Figure 3: Distribution of NAFLD patients by fibrosis staging (N=80)

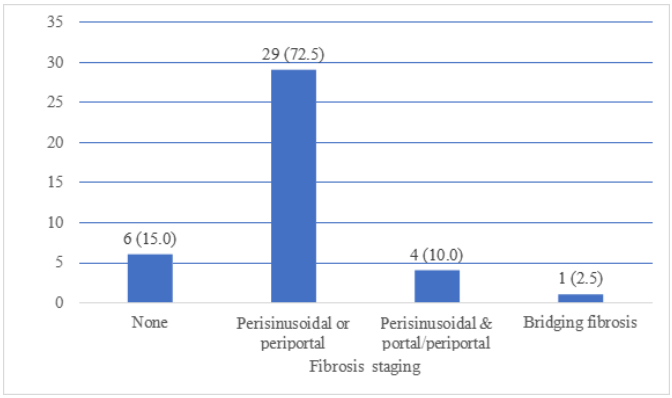

Figure 3 depicts that among the participants with NAFLD, majority (72.5%) were suffering from perisinusoidal or periportal stage of NAFLD.

Table 3: Comparison of serum ethanol level among NASH and Non-NASH patients (N=40)

Serum Ethanol (mg/dl)	NAFLD		P value
	NASH	Non-NASH	
Mean ± SD	1.91±1.22	0.93±1.38	
Median (range)	2 (0-4)	0 (0-5)	0.018[S]

Table 3 is showing that significant difference of serum ethanol level was found among NASH (1.91±1.22 mg/dl) and Non-NASH (0.93±1.38 mg/dl) patients (p value: 0.018).

Table 4: Risk factor analysis for NASH (n=40)

Table 4 is showing that patients with Serum Ethanol level of ≥1.50 (mg/dl) were significantly 7.877 times at risk (95% CI: 1.49-41.383) of developing NASH, and patients with Serum triglyceride level of ≥150 mg/dl were non significantly 2.625 times at risk (95% CI: 0.400-17.224) of developing NASH. Patients with HOMA-IR level of ≥1.78 were non significantly 1.894 times at risk (95% CI: 0.337 -10.653) of developing NASH.

Attribute	Odds ratio	95% Confidence Interval		P value
		Lower	Upper	
S. Triglyceride				

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<150 mg/dl(Ref. category)				
≥150mg/dl	2.625	0.400	17.224	0.315^{NS}
Serum Ethanol				
<1.50mg/dl(Ref. category)				
≥1.50mg/dl	7.877	1.499	41.383	0.015^{S}

Discussion:

Due to having interaction of genetic predisposition, NAFLD is a multifactorial disease, other causes like metabolic, inflammatory and environmental factors also influence the composition of the gut microbiome. The microbiome composition can be modified by dietary intake, leading to gut microbiome dysbiosis, especially when these diets are rich in saturated fats, fructose, and animal-based foods. Ethanol is an end-product of dietary carbohydrate fermentation by intestinal bacterial flora. Under the anaerobic conditions in the colon, bacterial metabolism of pyruvate (a product of carbohydrate breakdown) generates acetaldehyde, which is further reduced to form ethanol. This process is favored when there is intestinal overgrowth of bacteria or yeast or if there is excessive consumption of dietary carbohydrate. Moreover, genetic deficiencies of human enzymes that metabolize bacterially generated acetaldehyde to acetate accentuate the endogenous production of ethanol.

This observational study was carried out to evaluate the relationship of serum ethanol level with different stages of NAFLD patients and healthy individuals. Forty NAFLD patients underwent percutaneous liver biopsy and forty healthy individuals were included. Liver biopsies were categorized as either NASH (n=11) or non-NASH (n=29) NAFLD. This study finding reveals statistically significant higher serum ethanol level in NASH patients than non-NASH patients and healthy individuals. This study also reveals positive correlation between serum ethanol level and hepatic fibrosis stages.

In our study, the mean age of the study population was 39.51±10.27 years with a minimum of 22 years and maximum age of 60 years, most of the NAFLD patients (42.5%) were 31 to 40 years of age. It was probably due to lower participation in sports and exercise-related activity, the habit of higher consumption of soft drinks, and fast foods containing refined carbohydrates and saturated fats. Similar findings were also observed by Agrawal et al. (2009) who reported mean age of 42.7±10.09 with the range of 25 - 62 years [11]. On evaluating the prevalence and risk factors of NAFLD in Bangladesh Alam et al (2018) found the mean age of the participants was

34.21 years ±12.66, ranging from 18 to 85 years [12].

In a prospective follow-up study, Paul et al (2018) also observed the mean age of male and female NAFLD patients were 34.25±6.47 years and 33.54±6.14 years respectively [13]. These findings were in accordance with this study.

They also found serum ethanol concentration was not different between healthy subjects and obese patients. In this study there was also significant difference (p value: 0.018) of serum ethanol level between NASH and Non-NASH patients, Volynets et al. (2011) found blood alcohol levels were higher in patients with NAFLD than in controls. This discrepancy may be due to least number of NASH patients found in NAFLD group in current study [14].

Pulzi et al. (2011) showed total cholesterol (TC) ≥200 mg/dL, alanine aminotransferase (ALT) ≥30, AST/ALT ratio (AAR)≤ 1, gamma glutaril-transferase (GGT) ≥30 U/L and abdominal US, compatible with steatosis, had

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association with NASH group [15]. They confirmed that the combination of biochemical and imaging results improved accuracy to 84.4% the recognition of NASH (sensitivity 70%, specificity 88.6%, NPV 91.2%, PPV63.6%). An earlier study by Chen et al. (2020) also demonstrated endogenous ethanol produced by intestinal microbiota is an important factor causing liver lipid accumulation and thereby providing new insights into the etiology of NAFLD [16]. In this study showed that patients with serum ethanol level of ≥1.50 (mg/dl) were significantly 7.877 times at risk (95% CI: 1.49-41.383) of developing NASH, and patients with serum triglyceride level of ≥150 mg/dl were non significantly 2.625 times at risk (95% CI: 0.400-17.224) of developing NASH.

Conclusion:

Serum ethanol level was higher in NASH patients in comparison to non-NASH patients and healthy individuals. Also, positive correlation between NAS score and serum ethanol level was observed. However, this present study was a single center study only for 8 months, so variability of findings in different centers was possibility. Result may not reflect the exact picture of the whole country.

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