

# Assessment of Fatty Liver Diseases Based on Serum Ck-18 Fragment, Gamma Glutamyl Transferase and Aspartate Aminotransferase /Alanine Aminotransferase Ratio

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## Abstract

**Background:** Nonalcoholic fatty liver disease (NAFLD) is a serious clinical problem because of its worldwide distribution and potential adverse sequel. The global prevalence of NAFLD is increasing gradually. NAFLD can be estimated by both invasive and non-invasive methods.

**Objective:** The aim of this study was to determine the role of serum cytokeratin-18 (CK-18) fragment, gamma glutamyl transferase (GGT) and aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR) in the assessment of different types of NAFLD patients especially NASH.

**Methods:** This cross sectional study was conducted in the Department of Clinical Pathology, Bangabandhu Sheikh Mujib Medical University. Forty patients who fulfilled the criteria of NAFLD were enrolled in this study. Serum CK-18 fragment level was measured by enzyme linked immune sorbent assay (ELISA) method. Serum gamma glutamyl transferase (GGT) and aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR) reports were collected from patient's file. Biopsy material was sent to the Department of Pathology, BSMMU for complete histopathological examination.

**Results:** Positive and significant co-relation was found between serum CK-18 fragment with NAS whereas positive but not significant correlation was seen between serum GGT and NAS and negative and not significant correlation was found between serum (AAR) and NAS.

**Conclusion:** Serum CK-18 fragment level may be used for assessing NASH in NAFLD patients.

**Key words:** serum CK-18 fragment level; gamma glutamyl transferase level; nonalcoholic fatty liver disease

## Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the common forms of chronic liver diseases [1]. It is currently estimated that the global prevalence of NAFLD is one billion [1]. This prevalence is increasing gradually which will become the major economic burden [2, 1]. In a study Nonalcoholic steatohepatitis (NASH) was observed in 42.4% of NAFLD cases in Bangladesh [3]. Histologically nonalcoholic fatty liver disease is classified into nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH) [4]. Among them NASH may be present in one third of NAFLD [5]. Nonalcoholic steatohepatitis is a potentially serious condition. The prevalence of NAFLD is 15% in Asian population and 20 to 30% in United States [6]. It is thought to be the hepatic manifestation of metabolic syndrome [7]. The detection of CK18 fragment can differentiate NASH from NAFL or simple steatosis in this way.

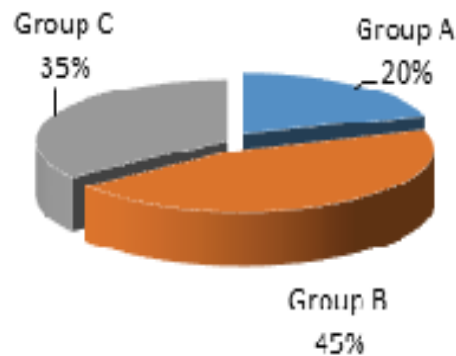
## Materials and Methods

The research protocol of this study was approved by the Ethical Institutional Review Board of BSMMU, Dhaka prior to the commencement of it. This cross sectional study was conducted in the Department of Clinical Pathology, in collaboration with Department of Hepatology and Department of Pathology, in Bangabandhu Sheikh Mujib Medical University, Dhaka from March' 2014 to February' 2015. 40 NAFLD patients of above 18 years old ages of both sexes who admitted in the Department of Hepatology were included in the study. Patients having history of alcoholism more than 210gm/wk for male and 140gm/wk for female, any condition like decompensated cirrhosis of liver, infected with hepatitis B and C virus infection, drug induced fatty liver, hepatocellular carcinoma, autoimmune liver diseases,

hemochromatosis, wilson's disease and patients with hypothyroidism were excluded from the study. Total NAFLD patients were divided into three groups according to the NAFLD activity score (NAS); NASH Clinical Research Network histological scoring system which is based on histopathological examination [8]. After taking informed written consent, a careful history was taken and the detailed information was recorded by the investigator in a predesigned questionnaire. The serum separation, storing and ELISA procedure were done in the Clinical Pathology Department, BSMMU. CK-18 fragment level is measured by Enzyme linked Immuno Sorbent Assay (ELISA) based on double antibody sandwich technology. Hematoxylin & Eosin and Masson's Trichome stains were used and evaluated using NASH Clinical Research Network histological scoring system to diagnosis the NAFLD. All data were recorded systematically in a preformed data collection sheets and expressed as mean±SD. For all statistical tests we considered p value <0.05 as statistically significant. Statistical analyses of the results were obtained by Pearson's correlation coefficient test. All statistical computations were performed by using window based computer software devised with Statistical Packages for Social Sciences (SPSS 17.0).

## Results

A total of 40 patients with NAFLD were included in this study. In this study, histopathology was considered as gold standard to divide the patients into 3 groups based on Nonalcoholic fatty liver disease activity score (NAS), including Group A : 1 to 2 = simple fatty liver disease; Group B: 3 or 4 = Borderline NASH Patients and Group C: 5 to 8 = Definite NASH.



**Figure 1: Distribution of study population of NAFLD (n=40)**

**Table I: Age distribution of the study patients according to groups of nonalcoholic fatty liver disease (NAFLD) (n=40)**

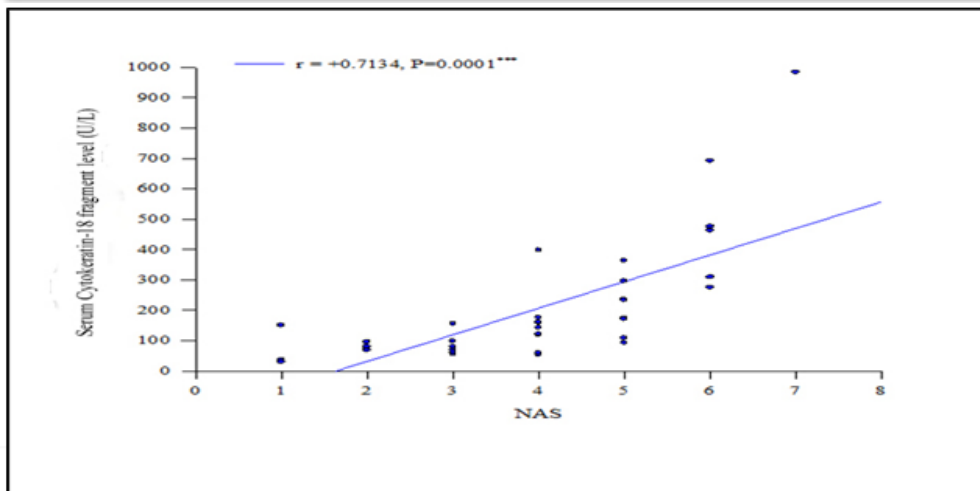
Age (years)	Group A No. (%)	Group B No. (%)	Group C No. (%)	<i>p</i> value
26-40	4 (50.0)	11 (61.1)	7 (50.0)	0.710 <sup>ns</sup>
41-50	2 (25.0)	6 (33.3)	5 (35.7)	
51-60	2 (25.0)	1 (5.6)	2 (14.3)	
<b>Total</b>	<b>8 (20.0)</b>	<b>18 (45.0)</b>	<b>14 (35.0)</b>	
Mean±SD	42.63±10.07	40.06±7.88		0.492 <sup>ns</sup>
Range	30.00-56.00	26.00-56.00		
Mean±SD	42.63±10.07		43.43±8.97	0.836 <sup>ns</sup>
Range	30.00-56.00		30.00-60.00	
Mean±SD		40.06±7.88	43.43±8.97	0.285 <sup>ns</sup>
Range		26.00-56.00	30.00-60.00	

Chi-square test/ANOVA (PostHoc) test, ns = not significant

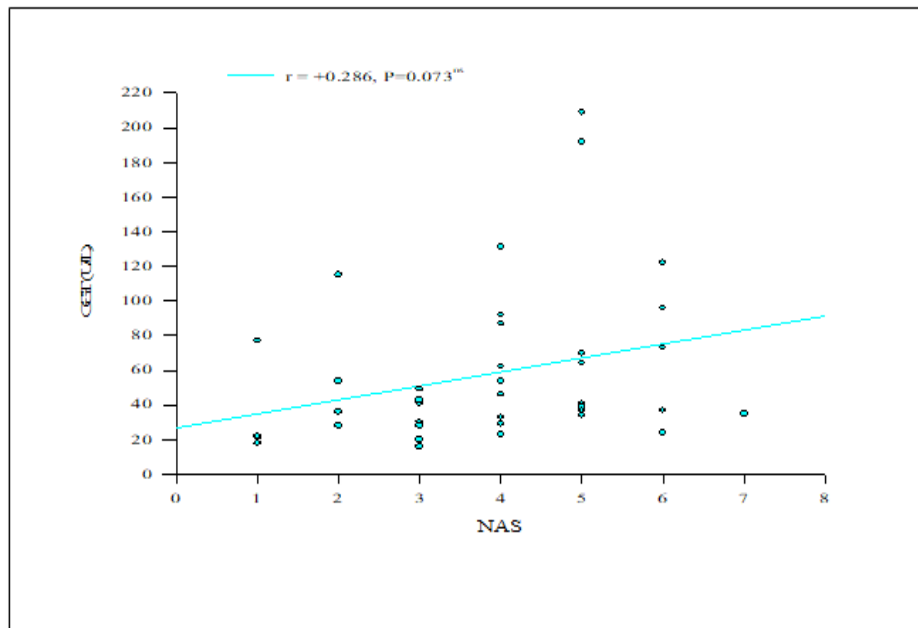
**Table II: Gender distribution of the study patients according to groups of NAFLD (n=40)**

Gender	Group A No. (%)	Group B No. (%)	Group C No. (%)	<i>p</i> value
Male	3 (37.5)	4 (22.2)	5 (35.7)	0.622 <sup>ns</sup>
Female	5 (62.5)	14 (77.8)	9 (64.3)	
<b>Total</b>	<b>8 (20.0)</b>	<b>18 (45.0)</b>	<b>14 (35.0)</b>	

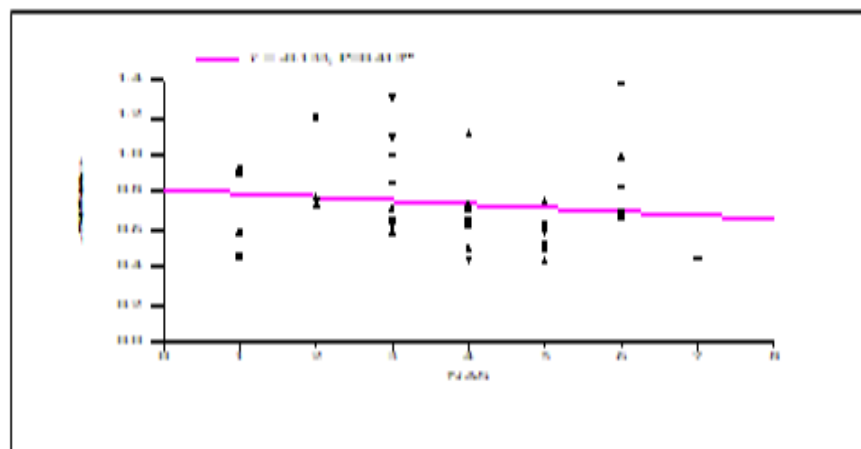
Chi-square test, ns = not significant



**Figure 2:** Showing positive correlation between NAS and cytokeratine-18 fragment level ( $r = +0.7134, p=0.001$ )



**Figure 3:** Showing positive correlation between NAS and GGT ratio ( $r = +0.286$ ,  $p > 0.05$ )



**Figure 4:** Showing negative correlation between NAS and AST-ALT ratio ( $r = -0.133$ ,  $p > 0.05$ )

## Discussion

This cross sectional study was conducted in the department of Clinical Pathology in collaboration with department of Hepatology, BSMMU, Dhaka. In this study, we investigated 40 NAFLD patients who fulfilled the inclusion criteria of the study. In this study, out of 40 patients of NAFLD, histopathological examination showed that 8 (20%) patients had simple fatty liver (Gr-A), 18 (45%) had borderline NASH (Gr-B) and 14 (35%) had NASH (Gr-C). Regarding the age, in this study, out of 40 patients, the mean ( $\pm$ SD) age of Group A (=simple fatty liver) patients was  $42.63 \pm 10.07$  (range 30-56) years, Group B (=borderline diagnosis) was  $40.06 \pm 7.88$  (range 26-56) years and Group C (=NASH) was  $43.43 \pm 8.97$  (range 30-60) years. In this study, patients with NASH were found at 30-60 years age group. Alam et al. mentioned that NASH affects the population with mean age  $40 \pm 9.7$  with range from 30-50 of Bangladesh [3]. Aktas et al. observed that the mean age of NAFLD patients was  $47 \pm 12$  (years) with range from 35-59 in his study [9]. Papatheodoridis et al. showed that mean age of NASH patients in his study was  $48 \pm 13$  (35-61).<sup>10</sup> Our study findings were similar with these studies. Analysis of gender distribution showed out of 40 NAFLD patients

12 were male and 28 were females. Male female ratio was 1:2:3. Out of 8 patients of Group A, 3(37.5%) were male and 5(62.5%) were female; out of 18 patients of Group B, 4(22.2%) were male and 14(77.8%) were female; and out of 14 Group C patients, 5(35.7%) were male and 9(64.3%) were female. In this study females were predominant among three Groups. Alam et al. observed that female were predominant in NAFLD patients in Bangladesh [3]. Aida et al. also noticed female predominance in NAFLD patients in Japan [11]. Our study findings were consistent with these studies. In this study, Pearson's correlation-coefficient (r) test was performed to compare the relationship between the levels of CK-18 fragment with NAFLD Activity Score (NAS). We observed a positive correlation between GGT and NAS but not significant. Pearson's correlation coefficient was  $+0.286$ ,  $p > 0.05$ . Feldstein et al., Papatheodoridis et al. and Grigorescu et al. also found no significant correlation between GGT and NAS [8,10, 12]. So, our study was consistent with these studies. In the present study, we found a negative correlation between AST-ALT ratio and NAS, not significant Pearson's correlation coefficient was  $-0.133$ , ( $p > 0.05$ ). In the study

Feldstein et al. there was also no significant correlation found between AST-ALT ratio and NAS [8]. This finding supported our study.

## Conclusion

Our data revealed that there was statistically significant correlation between serum CK-18 fragment level with different types of NAFLD whereas, serum GGT and ALT-AST ratio were not significant statistically. In this aspect, we concluded that Serum CK-18 fragment level can be used for early detection, reduce progression, assessment of the prognosis of NASH in NAFLD patients.

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