

Role of Serum Ck-18 Fragment, Alanine Transaminase and Aspartate Transaminase Level in the Assessment of Fatty Liver Diseases

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Abstract:

Background: Nonalcoholic fatty liver disease is one of the commonest chronic diseases of liver. Nonalcoholic steatohepatitis (NASH) becomes a serious public health problem worldwide. Non-invasive markers may play an important role in the diagnosis of

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different types of nonalcoholic fatty liver disease (NAFLD) especially NASH.

Objective: *The aim of this study was to determine the role of serum cyokeratin-18 (CK-18) fragment, Alanine transaminase (ALT) and Aspartate transaminase (AST) level 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 recently. Forty patients who fulfilled the criteria of NAFLD were enrolled in study. Serum CK-18 fragment level was measured by enzyme linked immune sorbent assay (ELISA) method. Serum ALT and AST reports were collected from patient's file. Biopsy material was sent to the Department of Pathology, BSMMU for complete histopathological examination.*

Results: *Pearson's correlation coefficient were done ($r=0.713$, $p=0.001$); ($r=0.347$, $p=0.028$) and ($r=0.310$, $p=0.051$) between serum CK-18 fragment, ALT and AST level with NAFLD activity score (NAS). Positive and significant correlation was found between serum CK-18 fragment and ALT level with NAS whereas positive but not significant correlation was seen between serum AST and NAS. Serum CK-18 fragment level was more significant than ALT level. Binary multivariate logistic regression analysis was performed to determine independent predictors of NASH. Serum CK-18 fragment ($p=0.003$) and ALT ($p=0.045$) were significantly associated with the presence of NASH. Serum CK-18 fragment level ($p=0.003$) was more significant than ALT level ($p=0.045$) to predict the NASH.*

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

Key words: Serum CK-18 fragment level, Alanine transaminase (ALT), Aspartate transaminase (AST), Nonalcoholic fatty liver disease (NAFLD), Nonalcoholic steatohepatitis (NASH), Non alcoholic fatty liver disease activity score (NAS)

INTRODUCTION

Nonalcoholic fatty liver disease is one of the commonest chronic diseases of liver¹. The prevalence of NAFLD is 15% in Asian population and 20 to 30% in United States². As this number is increasing, it will become the global burden in both rich and poor countries³. Simple hepatic steatosis is usually benign condition. But NASH may progress to hepatic fibrosis, cirrhosis, hepatocellular carcinoma and hepatic failure⁴. Nonalcoholic steatohepatitis (NASH) threatens as it becomes a serious public health problem worldwide⁵. In a study NASH was observed in 42.4% of NAFLD cases in Bangladesh⁶. Liver biopsy is the gold standard for diagnosis of NASH. It has several disadvantages. In our country biopsy is not possible in all hospital due to lack of expert manpower. Moreover, it is invasive, expensive, associated with various complications and sampling errors. It is also contraindicated in some patients. Due to heterogenous distribution of the lesions there remains a scope of errors. For these reasons, it is poorly suited as a diagnostic tool^{7,5}. Development of serum markers offers an attractive, cost effective alternative to liver biopsy for both patients and physicians. Nowadays, varieties of non invasive tests have been focused as potential alternatives to liver biopsy. These includes laboratory tests like liver function tests such as measurement of aminotransferases level (ALT and AST levels), RDW (red cell distribution width) and MPV (mean platelet volume), markers of extracellular matrix remodeling and radiologic imaging studies^{8,9,4}.

Alanine aminotranferase (ALT) and aspartate aminotransferase (AST) are the indicators of hepatocellular injury. ALT is found in the cytosol of the hepatocytes. In hepatocytes it transfers amino groups from alanine to ketoglutarate. ALT is mostly specific to liver but it can also be

found in blood during muscle injuries or inflammation. The major site of AST is mainly mitochondria in hepatocyte¹⁰. Elevated serum ALT and AST levels are the primary abnormality seen in patients with NAFLD. These levels are higher in patients with NASH than NAFL¹¹. On the other hand, Cytokeratin is the major intermediate filament protein in cells. It forms cytoplasmic network. CK-18 maintains normal cellular and mitochondrial structure. It is also involved in apoptosis¹².

In NASH several mechanisms of cell death follow including apoptosis, necrosis, necroptosis (both necrosis and apoptosis) and autophagy¹. In apoptosis the enzyme caspases become catalytically active and the effector caspases cleave CK-18¹³. Caspase-cleaved CK18 fragment is released into the extracellular compartment⁵. Then it can be detected in the blood of patients with NASH by using a monoclonal antibody, M30¹⁴, whereas this CK-18 fragment concentration is not increased in patients with simple fatty liver⁵. This assessment differentiates NASH from NAFL or simple steatosis. Thus CK18 fragment concentration reflects the pathogenesis of NASH and refines the assessment of disease¹⁵

MATERIALS AND METHODS

This cross sectional study was conducted at the Department of Clinical Pathology, in collaboration with Department of Hepatology and Department of Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka over the period from March' 2014 to February' 2015. 40 NAFLD patients who admitted in the Department of Hepatology were enrolled in the study. Total NAFLD patients were divided into three groups according to the NAFLD activity score (NAS); NASH Clinical Research Network histological scoring system¹⁶ which is based on histopathological examination¹⁶. These groups are: Group A

→ Non NASH which includes simple fatty liver disease patients. Here 8 patients were included. Group B → Borderline patients. Here 18 patients were included and Group C → Definitive NASH patients. Here 14 patients were included. 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 (oral contraceptive pill, amiodarone, methotrexate and tamoxifen), hepatocellular carcinoma, autoimmune liver diseases, hemochromatosis, wilson's disease and patients with hypothyroidism were excluded from the study. After taking informed written consent, a careful history and the details information were recorded in a predesigned questionnaire. Serum CK-18 fragment level is measured by enzyme linked immune sorbent assay (ELISA). Serum ALT and AST reports were collected from patient's file. Needle liver biopsy was done by Hepatologist. Biopsy material was fixed in 10% formalin and sent to the Department of Pathology, BSMMU for complete histopathological examination. Haematoxyline & Eosin and Masson's Trichome stain were done and evaluated using NASH Clinical Research Network histological scoring system to diagnosis the NAFLD. All data was recorded systematically in a preformed data collection sheet and expressed as mean \pm 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 and Binary multivariate logistic regression analysis. All statistical computations were performed by using window based computer software devised with Statistical Packages for Social Sciences (SPSS 17.0). Prior to the commencement of this study, the research protocol was approved by the Ethical Institutional Review Board of BSMMU, Dhaka.

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. Table III shows age distribution of the study patients according to groups of nonalcoholic fatty liver disease (NAFLD).

Figure 01: Distribution of study patients (n=40).

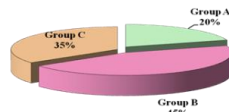


Figure 3 Groups of NAFLD (n=40)

Figure 01 shows that out of 40 patients, 8 (20%) had simple fatty liver disease (Group A), 18 (45%) borderline NASH (Group B) and 14 (35%) had NASH (Group C).

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 value</i> |
|--------------|-----------------|------------------|------------------|---------------------|
| 26-40 | 4 (50.0) | 11 (61.1) | 7 (50.0) | |
| 41-50 | 2 (25.0) | 6 (33.3) | 5 (35.7) | 0.710 ^{ns} |
| 51-60 | 2 (25.0) | 1 (5.6) | 2 (14.3) | |
| Total | 8 (20.0) | 18 (45.0) | 14 (35.0) | |
| Mean±SD | 42.63±10.07 | 40.06±7.88 | | 0.492 ^{ns} |
| Range | 30.00-56.00 | 26.00-56.00 | | |
| Mean±SD | 42.63±10.07 | | 43.43±8.97 | 0.836 ^{ns} |
| Range | 30.00-56.00 | | 30.00-60.00 | |
| Mean±SD | | 40.06±7.88 | 43.43±8.97 | 0.285 ^{ns} |
| Range | | 26.00-56.00 | 30.00-60.00 | |

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

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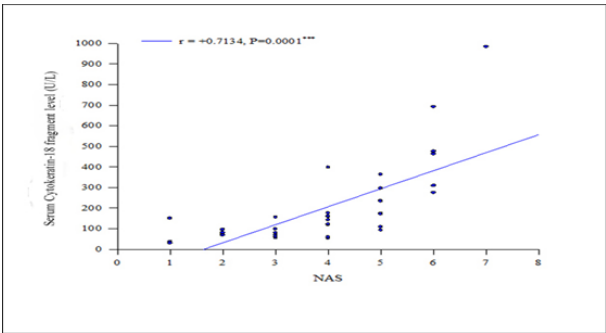
Table II: Gender distribution of the study patients according to groups of NAFLD (n=40)

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

Chi-square test, ns = not significant

Table II shows gender distribution of NAFLD patients. Gender distribution of overall patients showed 70% female patients and 30% male patients. Female predominance was observed in all study groups. 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. Gender distribution of patients according to type of NAFLD, statistically did not show significant variation ($p>0.05$).

Figure 02: Correlation between serum CK-18 fragment level and NAS (n=40)



In Figure 02 positive and highly significant correlation ($r=0.7134$, $p<0.001$) was found between serumCK-18 fragment

level and NAS. Serum CK-18 concentration was measured in 40 NAFLD patients and expressed as U/L. Types NAFLD was categorised by Nonalcoholic fatty liver disease Activity Score (NAS) which was expressed numerically (0-8).

Figure 03: Correlation between NAS and ALT level (n=40)

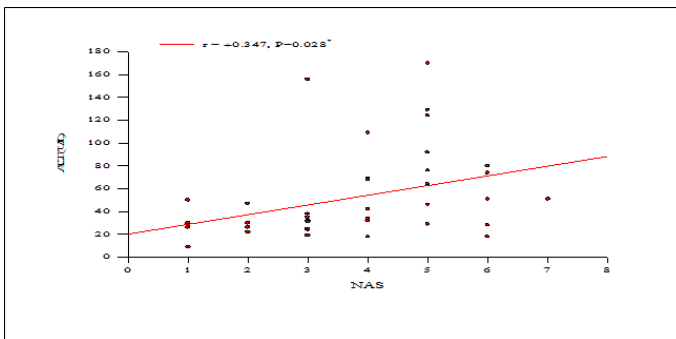


Figure 03: Showing positive correlation between NAS and ALT level ($r = 0.347, p < 0.05$)

In Figure 03 ALT levels of all patients with NAFLD were expressed in U/L and correlated with NAS. Positive and significant ($r = 0.347, p < 0.05$) correlation was found between ALT and NAS.

Figure 04: Correlation between NAS and AST level (n=40)

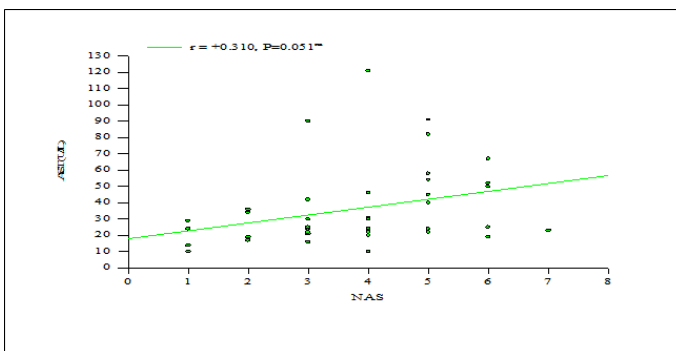


Figure 04: Showing positive correlation between NAS and AST level (r=0.310, p>0.05)

In Figure 04 AST levels of all patients with NAFLD were expressed in U/L and correlated with NAS. Positive but not significant correlation (r=0.310, p>0.05) was found between AST and NAS.

Table III: Binary multivariate logistic regression analysis for serum CK-18 fragment, ALT and AST for the diagnosis of NASH (n=40)

| | B | S.E. | Wald | df | Sig. | Exp(B) |
|----------------|--------|-------|--------|----|-------|--------|
| ALT | 0.066 | 0.033 | 4.017 | 1 | 0.045 | 1.069 |
| AST | -.050 | 0.048 | 1.083 | 1 | 0.298 | 0.951 |
| CK-18 fragment | 0.020 | 0.007 | 8.679 | 1 | 0.003 | 1.021 |
| Constant | -6.342 | 1.987 | 10.185 | 1 | 0.001 | 0.002 |

Table III shows binary multivariate logistic regression analysis to determine the independent predictors of NASH. Statistical comparison between serum CK-18 fragment, ALT and AST reveals that serum CK-18 fragment (p=0.003) and ALT (p=0.045) were significantly associated with the presence of NASH whereas AST (p=0.298) was not significantly associated with NASH. Serum CK-18 fragment level (p=0.003) was more significant than ALT level (p=0.045) to predict the NASH.

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 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 ± 10.07 (range 30-56) years, Group B (= borderline diagnosis) was 40.06 ± 7.88 (range 26-56) years and Group C (=NASH) was 43.43 ± 8.97 (range 30-60) years. In this study, patients with NASH were found at 30-60 years age group. Alam et al. (6) found that NASH affects the population with mean age 40 ± 9.7 with range from 30-50 of Bangladesh. Aktas et al. (9) observed that the mean age of NAFLD patients was 47 ± 12 (years) with range from 35-59 in this study. Papatheodoridis et al. (17) showed that mean age of NASH patients in his study was 48 ± 13 (35-61). 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., (2013) observed that female were predominant in NAFLD patients in Bangladesh. Aida et al. (18) also found female predominance in NAFLD patients in Japan. 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 found that level of serum CK-18 fragment was increased with increasing NAS. There was strongly positive and significant correlation found between NAS and serum CK-18 fragment

($r=0.713$ and $p<0.05$). In the study of Aida et al. (18), Feldstein et al. (5), and Tsutsui et al. (19), Papatheodoridis et al. (17) a significant positive correlation between serum CK-18 fragment levels and NAS were found. So our study was similar to these studies.

In this study, a positive and significant correlation was found between NAS and ALT. Pearson's correlation coefficient was 0.347, $p<0.05$. Our study finding was similar with previous study done by Yilmaz et al. (14), Grigorescu et al. (15).

In the present study, we found a positive correlation between AST and NAS but not significant. Pearson's correlation coefficient was 0.310, $p>0.05$. Papatheodoridis et al., (17), Grigorescu et al. (15), Alam et al. (6) also, found no significant correlation between AST and NAS. This finding was similar with our study.

In our study binary multivariate logistic regression analysis was performed to determine the independent predictors of NASH. Statistical comparison between serum CK-18 fragment, ALT and AST reveals that serum CK-18 fragment ($p=0.003$) and ALT ($p=0.045$) were significantly associated with the presence of NASH whereas AST ($p=0.298$) was not significantly associated with NASH. Serum CK-18 fragment level ($p=0.003$) was more significant than ALT level ($p=0.045$) to predict the NASH. Feldstein et al. (5), Tsutsui et al. (19) and Wieckowska et al. (20) also observed that serum CK-18 fragment was independently associated with NASH. So our study findings were consistent with their study findings.

According to our study findings, serum CK-18 fragment concentration and serum ALT level are significantly higher in patients with nonalcoholic steatohepatitis. There is strong positive relationship between serum CK-18 fragment and ALT level with different types of NAFLD. Serum CK-18 fragment level is more significant than serum ALT level. Serum AST

level did not correlate with NAS. Our data indicates that serum CK-18 fragment level may be useful for a guideline for presenting NASH in NAFLD patients.

CONCLUSION

Our study revealed that there was statistically significant correlation between serum CK-18 fragment and ALT level with different types of NAFLD. Serum CK-18 fragment level is more significant than serum ALT level. Serum CK-18 fragment level can be used for the assessment of NASH in NAFLD patients.

Acknowledgement

Authors of this study are thankful to the authority of the Department of Hepatology, BSMMU, Department Pathology, BSMMU and the Department of Clinical Pathology, BSMMU, for their nice cooperation during sample collection, laboratory procedure and also thankful to the study subjects for their active and enthusiastic participation.

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