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Original Articles

Comparison of Serum C-Reactive Protein (CRP) Level Between Non-Alcoholic Fatty Liver Disease (NAFLD) Cases and Healthy Adults: A Cross-sectional Analytical Study

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Abstract

Background: *Non-alcoholic fatty liver disease (NAFLD) encompasses the simple steatosis to more progressive steatosis with associated hepatitis, fibrosis and cirrhosis. Though accurate diagnosis of simple fatty liver (SFL) and non-alcoholic steatohepatitis (NASH) can be made by liver biopsy, it is not feasible to be done in all NAFLD patients. Relationship of C-reactive protein (CRP) with NAFLD was well documented in many populations, but comprehensive data is lacking in Bangladeshi people.*

Method: *This case-control study was conducted at the Medicine, Gastroenterology and Hepatology departments in Dhaka Medical College Hospital from March to August 2018 involving 30 patients with NAFLD as case and apparently healthy 30 individuals as control. Abdominal ultrasound to detect fatty liver and serum CRP level estimation by nephelometry were performed for each subject to compare between two groups.*

Result: *Majority of the NAFLD cases and healthy controls were female (70% and 60% respectively). Mean age was 47.53±9.69 year in NAFLD group and 46.03±8.44 year in healthy controls. NAFLD cases had significantly higher mean serum CRP concentration (6.27±1.80) mg/dl than that of healthy controls (3.94±2.16) mg/dl (p<0.001). Overweight/obesity and dyslipidaemia were found as significant risk factors for NAFLD compared to the healthy control group (p<0.05).*

Conclusion: *NAFLD cases had significantly higher CRP than the control group. But, to find out the association of increasing CRP level with the severity of NAFLD and to establish it as a diagnostic tool, further extensive studies are recommended.*

Keywords: *Non-Alcoholic Fatty Liver Disease (NAFLD), C-Reactive Protein (CRP), Steatosis, Metabolic syndrome*

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Introduction:

Non-alcoholic fatty liver disease (NAFLD) encompasses the simple deposition of adipose tissue in the liver to more progressive steatosis with associated hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) in some cases.[1] In general, non-alcoholic fatty liver (NAFL) and non-

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alcoholic steatohepatitis (NASH) are collectively called NAFLD. NAFL is characterized by liver steatosis involving more than 5% of parenchyma, with no evidence of hepatocyte injury.[2] Whereas, NASH is defined by histologic terms as a necro-inflammatory process whereby the liver cells become injured in a background of steatosis.[1,2] Insulin resistance appears to be the centre for induction in the development of NAFLD. Therefore, NAFLD is considered to be the hepatic manifestation of metabolic syndrome.[3] In terms of epidemiology, a precise and reliable occurrence rate of NAFL/NASH is not currently available due to extreme variations in study parameters and available testing tools.[1,4] Several estimates suggest that the incidence of NAFLD to be 20%-30% in Western countries and 5%-18% in Asia.[4,5] Sedentary lifestyle, urbanization, and over-nutrition were responsible for this growing incidence of NAFLD.[6] The prevalence of NAFLD for Asian countries was estimated to be 27.4% (95% CI 23.3%–31.9%) and in the general Japanese population ranges from 24.6% to 29.7%,[7,8] similar to that in China and Korea. In India, the prevalence ranges from 5 to 28%. Moreover, a study from India showed that NAFLD accounts for about 63% of all cryptogenic cirrhosis cases.[9] In Bangladesh, the prevalence of NAFLD in general population is 4% to 18.4%,[10] which jumps up to 49.8% in diabetic patients. About 40% of them are non-alcoholic steatohepatitis (NASH). The risk of progression to cirrhosis and HCC will be 15%-19% in the next 5 to 10 years. Such circumstance warrants early identification by modern investigation techniques.[10,11]

The most challenging fact for diagnosing either NAFLD or NASH is the asymptomatic nature of the disease.[12] Unfortunately, many individuals may have entirely normal liver enzymes, even with significantly advanced steatosis.[12,13] A study by Fracanzani et al. showed that NASH was diagnosed in 59% and 74% of the patients with normal and increased Alanine transaminase, respectively.[14] Patients with normal waist circumference with milder metabolic alterations may have NASH, even fibrosis. It means, visceral obesity is not a major determinant to predict the severity of liver damage. Hence, it is necessary to diagnose NAFLD at the earlier stage, as timely taken measures can halt the progression. Liver biopsy is still the gold standard of diagnosis, but it is not feasible to perform this routinely. Therefore, various non-invasive serum as well as radiological markers have been proposed; and C-Reactive Protein (CRP) is one of them.[13,15,16]

CRP, a major acute phase response protein mainly produced in the liver acts as a marker of systemic inflammation in clinical practice. As inflammatory process is almost inevitable to NAFLD, therefore change in CRP level is also a consequence. CRP is a reliable, and inexpensive serum marker which is extensively used for diagnosis and follow up of several morbidities. It has been shown that an elevated level of hs-CRP (a high sensitivity C-reactive protein) is a strong predictor of cardiovascular disease risk and type-2 diabetes. A strong relationship between elevated hs-CRP levels and the risk of metabolic syndrome was established in various studies.[17] Oruc et al.[16] revealed that serum CRP level was increased in NAFLD compared to controls, admitting no value in discrimination of steatohepatitis from simple steatosis. Yeniova et al.[18] also showed higher hs-CRP levels in patients with NAFLD as compared to the control group and found it a non-invasive marker and a strong predictor of NAFLD. There are minimal similar studies found in our country. Therefore, the study was planned to assess the level of CRP in NAFLD patients admitted to a tertiary care hospital in Bangladesh.

Materials and methods:

This case-control study was conducted at Department of Medicine, Gastroenterology, and Hepatology in Dhaka Medical College Hospital (DMCH) during the period from March to August 2018. The study was aimed to investigate Non-Alcoholic Fatty Liver Disease (NAFLD) and involved a sample population of 60 individuals, comprising 30 cases diagnosed with NAFLD and 30 apparently healthy controls.

The sampling technique employed was purposive convenient sampling, and the sample size was determined based on a 4% prevalence of NAFLD, resulting in a total of 60 participants. Inclusion criteria for cases encompassed both male and female patients newly diagnosed with NAFLD, aged over 18 years, and in good general health. Control group participants were apparently healthy adults who willingly participated.

Exclusion criteria involved a history of alcohol ingestion, previous gastrointestinal surgery, malignancy, liver diseases causing fatty liver, recent use of drugs inducing hepatic steatosis, and lack of willingness to provide consent. Ethical clearance was obtained from the Dhaka Medical College's ethical review committee, and informed written consent was secured from all participants. Data collection involved face-to-face interviews using a semi-structured questionnaire covering socio-demographic and co-morbidity informations.

Both case and control groups underwent evaluation through trans-abdominal ultrasonogram (USG) and C-reactive protein (CRP) level measurements. NAFLD diagnosis was based on specific ultrasonographic criteria, and serum CRP levels were assessed using nephelometry. Data processing

and statistical analysis were conducted using Microsoft Excel and SPSS version 21.0, respectively. Descriptive statistics, t-tests, chi-square tests, and ANOVA were employed as appropriate, with a significance level set at $p<0.05$. This comprehensive methodology ensures a systematic investigation into the prevalence and associated factors of NAFLD within the specified study population.

Results:

In this study, 30 cases of NAFLD and 30 healthy controls were taken. Mean±SD of age was 47.53±9.69 year in NAFLD group and 46.03±8.44 year in healthy controls. Majority of the NAFLD cases were aged between 41-50 year (50%). Among healthy controls 40% were in the age group 41-50 year. There was no statistically significant difference in age distribution between groups ($p>0.05$). Majority of the NAFLD group and healthy controls were female (70% and 60% respectively). There was no statistically significant difference in sex distribution between two groups ($p>0.05$). Half of the NAFLD cases came from rural area and another half from urban area. Among healthy controls, 60% came from urban area and 40% from rural area. Among healthy controls, 60% came from urban area and 40% from rural area. There was no statistically significant difference in residence distribution between groups ($p>0.05$). Most of the NAFLD patients were illiterate (36.7%). Among healthy controls, 30% were illiterate followed by another 30% completing primary education. There was no statistically significant difference in distribution of education between groups ($p>0.05$). Most of the NAFLD patients were housewives (30%) followed by service holder (13.3%) and businessman (13.3%). Among healthy controls, majority were housewives (53.3%) followed by day labourer (23.3%). The difference in distribution of occupation between groups was not statistically significant ($p>0.05$).

Table 1. *Baseline characteristics of study population (n=60)*

Variables	Subgroups	NAFLD cases (30)	Healthy controls (30)	*p* value
Age distribution(year)	21 – 30	2 (6.7%)	1 (3.3%)	
	31 – 40	4 (13.3%)	6 (20.0%)	
	41 – 50	15 (50%)	12 (40%)	0.78*
	51 – 60	7 (23.3%)	7 (23.3%)	
	>60	2 (6.7%)	4 (13.3%)	
	Mean±SD (year)	47.53±9.69	46.03±8.44	0.52**
	Min-Max (year)	29 – 65	28 – 65	
Sex	Male	9 (30%)	12 (40%)	0.41*
	Female	21 (70%)	18 (60%)	
Residence	Rural	15 (50%)	12 (40%)	0.41*
	Urban	15 (50%)	18 (60%)	
Education	Illiterate	11 (36.7%)	9 (30%)	0.41*
	Primary	6 (20%)	9 (30%)	
	SSC	5 (16.7%)	5 (16.7%)	
	HSC	4 (13.3%)	5 (16.7%)	
	Graduate & above	4 (13.3%)	2 (6.7%)	
Occupation	Housewife	15 (30%)	16 (53.3%)	0.60*
	Labourer	3 (10%)	7 (23.3%)	
	Farmer	3 (10%)	2 (6.7%)	
	Service holder	4 (13.3%)	3 (10%)	
	BusinessmanStudents	4 (13.3%)1 (3.3%)	2 (6.7%)0	
Risk factors	Hypertension	6 (20%)	6 (20%)	NA
	Diabetes	7 (23.3%)	5 (16.7%)	0.51*
	Overweight/obesity	23 (76.7%)	12 (40%)	0.004*
	Dyslipidaemia	14 (46.7%)	5 (16.7%)	0.012*
	Smoking	3 (10%)	4 (13.3%)	0.68*
BMI(kg/m^2)	Normal (18.5-22.9)	7 (23.3%)	18 (60%)	
	Overweight (23-24.9)	9 (30%)	7 (23.3%)	0.009*
	Obesity (≥25)	14 (46.7%)	5 (16.7%)	

* *p* determined by Chi-Square test **p determined by Student's *t* test

Among 30 patients of NAFLD, 53.3% had grade 2 fatty liver, followed by 33.3% having grade 3 and 13.3% having grade 1 fatty liver.

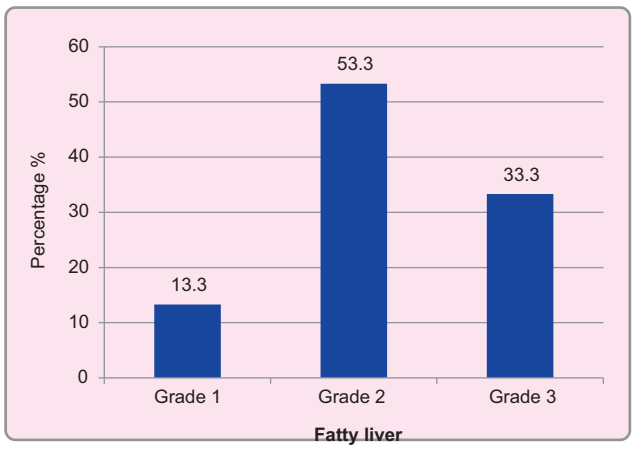

Figure 1: *Fatty liver grading among NAFLD cases (n=30)*

Higher grade of fatty liver was associated with significantly higher weight and BMI ($p<0.05$).

Table 2. *Characteristics of patients with non-alcoholic fatty liver according to its grade (n=30)*

Variables	Grade 1 (n=4)	Grade 2 (n=16)	Grade 3 (n=10)	*p* value*
Age (year)	44.0±10.42	49.69±9.11	45.50±9.69	0.42
Weight (kg)	47.50±2.88	59.18±8.78	67.20±3.55	<0.001
Height (m)	1.55±0.3	1.58±0.05	1.60±0.03	0.30
BMI (kg/m^2)	19.62±0.75	23.11±2.19	26.09±0.65	<0.001
CRP (mg/dl)	5.77±1.32	5.94±2.02	6.99±1.45	0.30

* *p* determined by ANOVA test

Table 3. *CRP level of study population (n=60)*

CRP	NAFLD cases (n=30)	Healthy controls (n=30)	*p* value
Raised	19 (63.3%)	7 (23.3%)	.002*
Not raised	11 (36.7%)	23 (76.7%)	
Concentration (mg/dl)	6.27±1.80	3.94±2.16	<0.001**

* *p* determined by Chi-Square test ** *p* determined by Student's *t* test

Considering the risk factors, NAFLD cases were significantly overweight/obese individuals than that of controls ($p<0.05$). Also, dyslipidaemia was significantly more prevalent among NAFLD patients than controls ($p<0.05$). Diabetes, hypertension and smoking habit were similarly distributed among groups ($p>0.05$).

NAFLD cases had significantly higher mean CRP concentration (6.27±1.80) mg/dl in blood than that of healthy controls (3.94±2.16) mg/dl ($p<0.05$). Among NAFLD cases, higher proportion of subjects had significantly raised CRP level ($p<0.05$).

Discussion:

In this study, people in the fourth to the fifth decade of age were more commonly presented with NAFLD with no specific male-female preponderance. There is no statistically significant finding in socio-demographic parameters of the cases. The majority population in both groups were illiterate, although the difference is insignificant. Moreover, there was no effect of occupation on their disease process in the study population. Overweight/obesity and dyslipidaemia were identified as risk factors in this study. Half of the patients in this study were found in grade 2, followed by grade 3 and

grade 1 fatty liver disease. A significant relationship ($p<0.05$) was noted between C-reactive protein (CRP) concentration in blood and the presence of non-alcoholic fatty liver disease (NAFLD). A limited number of other studies also found raised CRP in NAFLD.[16,19] CRP has a short life of around 18 hours. The elevation of serum CRP usually reflects its synthesis in response to a pathological process. Therefore, CRP is considered as a useful non-specific biochemical marker of chronic inflammation.[20] Oruc et al.[16] had the same conclusion in their study evaluating serum procalcitonin and CRP levels in non-alcoholic fatty liver disease. This response, however, alone has no diagnostic specificity. Hence, serial measurement of CRP may play a role in the clinical management and follow-up of NAFLD patients.[16]

In clinical trials, CRP elevation was related to metabolic syndrome and its components. Although the liver is the primary source of CRP production; adipose tissue, especially visceral fat, significantly contributes to CRP production. In the study conducted by Anty et al., CRP concentration was elevated in severely obese patients.[21] However, this elevation was moderate and not related to metabolic syndrome, diabetes, or steatohepatitis. In this study, grades of the fatty liver increased with increasing body mass index (BMI). Similar relationship was found in the study by Foroughi et al.[22]

The present study showed that CRP levels rise with the increasing grade of fatty liver in NAFLD cases. But it was not analysed in this study whether raised CRP is associated with the severity of NAFLD. However, Foroughi et al. found a significant association between CRP and increasing grades of fatty liver.[22]

Limitations of the study:

- This study was conducted in a single centre.
- Minimum sample size and short period of study.
- Long-term follow-up was beyond the scope.

Conclusion:

Serum C-reactive protein (CRP) levels were significantly higher in the cases of non-alcoholic fatty liver disease (NAFLD). Besides, increased body mass index and dyslipidaemia can be considered as two identified risk factors of NAFLD. However, to make CRP as a diagnostic tool for NAFLD requires large cohort study.

Conflict of Interest: There is no conflict of interest in this study.

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