SERUM HS-CRP LEVELS DO NOT INDICATE THE SEVERITY OF LIVER DAMAGE IN NON-ALCOHOLIC STEATOHEPATITIS PATIENTS WITH TYPE-2 DIABETES MELLITUS

Gönenç Kocabay MD,[1] Aysegül Telci,[2] Yıldız Tutuncu,[1] Betül Tiryaki,[3] Sevda Özel,[4] Uğur Çevikbaş,[5] Atilla Ökten,[6] İlhan Satman[1]

[1] Departments of Endocrinology and Metabolism, Faculty of Istanbul Medicine, Istanbul University, Istanbul, Turkey
[2] Department of Clinical Biochemistry, Faculty of Istanbul Medicine, Istanbul University, Istanbul, Turkey
[3] Department of Radiology, Faculty of Istanbul Medicine, Istanbul University, Istanbul, Turkey
[4] Departments of Public Health and Bioistatistics, Faculty of Istanbul Medicine, Istanbul University, Istanbul, Turkey
[5] Department of Pathology, Faculty of Istanbul Medicine, Istanbul University, Istanbul, Turkey
[6] Department of Gastroenterohepathology, Faculty of Istanbul Medicine, Istanbul University, Istanbul, Turkey

ABSTRACT

• ***Objective:*** *Non-alcoholic steatohepatitis (NASH) is a commonly encountered community health problem which is associated with obesity, insulin resistance and type 2 diabetes. There is no clinical or biochemical indicator predicting the prognosis of NASH. Therefore, it is not possible to predict the course of NASH in which patients may stay as a benign condition or in which it may progress to fibrosis or rarely cirrhosis. High sensitive C-reactive protein (hs-CRP) is a commonly used acute phase reactant which increases in even mild inflammatory states. In this study, we investigated the relationship between hs-CRP and inflammation in liver tissue in cases with NASH and type 2 diabetes mellitus.*

• ***Material and Method:*** *The study included 52 NASH cases with type 2 diabetes mellitus. NASH was diagnosed based on biochemical tests and ultrasound images of the liver. Each patient had a true cut liver biopsy. Histopathological evaluation of biopsy specimens were performed following Hematoxilen-Eosin and Mason Trichrome stains according to the Brunt Criteria. Fasting blood glucose (FBG), alkaline phosphatase (ALP), aspartat and alanine aminotransferase (AST and ALT), lactic dehydrogenase, gamma glutamyl transpeptidase (GGT), Hemoglobin (A1c) and hs-CRP levels were investigated. Insulin resistance was calculated using "Homeostasis Model of Assessment" (HOMA-IR) formula.*

• ***Results:*** *Serum levels of hs-CRP were tended to be higher as the severity of steatosis increased, however, did not reach to a statistical difference (3.6±3.4 mg/l in mild, 4.1±2.4 mg/l in moderate, and 6.0±4.4 mg/l in severe groups (Kruskal-Wallis variance analysis p= 0.18). Comparison of hs-CRP levels with and without inflammation or with and without fibrosis revealed similar tendency (respectively, p= 0.20 and p= 0.29). Hs-CRP was positively correlated with A1c, FBG, ALP, GGT and negatively correlated with AST. After adjustment of age and duration of diabetes, the above mentioned correlations remained same.*

• ***Conclusion:*** *Positive correlations between hs-CRP levels and cholestatic liver enzymes (ALP, GGT) suggested that peripheral inflammatory markers reflect the functional disorder. However, hs-CRP level did not seem to play a predictive role in progressive steatosis, inflammation or fibrosis.*

• ***Key Words:*** *Non-alcoholic steatohepatitis (NASH), type 2 diabetes mellitus, hs-CRP, liver enzymes, Brunt's classification system.* ***Nobel Med 2010; 6(2): 43-46***

ÖZET

TİP 2 DİYABETİK NON-ALKOLİK STEATOHEPATİTİ OLAN HASTALARDA SERUM YÜKSEK DUYARLIKLI CRP DÜZEYLERİ KARACİĞER HASARININ CİDDİYETİNİ GÖSTERMEZ

• **Amaç:** Non-alkolik steatohepatit (NASH), günümüzde sıklığı giderek artan, obezite, insülin rezistansı ve tip 2 diabetes mellitus ile ilişkilendirilen önemli bir sağlık sorunudur. Non-alkolik steatohepatitin prognozunu öngören klinik veya biyokimyasal belirteç yoktur. Bu nedenle, NASH seyrinde hangi hastaların iyi seyirli, hangilerinin nadir olsa da siroza ilerleyeceğini tahmin etmek mümkün değildir. Yüksek duyarlıklı C-reaktif protein (hs-CRP), hafif bir inflamasyonda bile yükselen, yaygın olarak kullanılan bir akut faz reaktandır.

Bu çalışmada, tip 2 diyabetik NASH hastalarında karaciğer dokusunda gelişen inflamasyon ile hs-CRP arasındaki ilişki incelendi.

• **Materyal ve Metod:** Çalışmaya, 52 tip 2 diyabetik NASH hastası alındı. NASH tanısı, biyokimyasal test ve ultrasonografik incelemeler ile konuldu. Her hastaya 'true cut' karaciğer biyopsisi yapıldı. Biyopsi materyalleri, Hematoksilen-Eosin ve Mason Trikrom boyamalarından sonra Brunt kriterlerine göre değerlendirildi. Açlık kan glukozu (AKG), alkalen fosfataz (ALP), aspartat ve alanin aminotransferaz (AST ve ALT), laktat dehidrogenaz, gama glutamil transpeptidaz (GGT), hemoglobin A1c (A1c) ve hs-CRP düzeyleri araştırıldı. İnsülin direnci, "Homeostasis Model of Assessmen" (HOMA-IR) formülü ile hesaplandı.

• **Bulgular:** Serum hs-CRP düzeyleri, steatoz derecesi arttıkça yükselme eğiliminde olmasına rağmen, anlamlılık sınırına ulaşmamıştır (3,6±3,4 mg/l hafif grupta, 4,1±2,4 mg/l orta grupta ve 6,0±4,4 mg/l ağır grupta (Kruskal-Wallis varians analiz, p=0,18). Hs-CRP düzeyleri, inflamasyonun ve fibrozun varlığı ile yokluğu karşılaştırıldığında benzerdi (sırasıyla, p=0,20 ve p=0,29). Hs-CRP; A1c, AKG, ALP, GGT ile pozitif korele iken, AST ile negatif korele bulundu. Yaş ve diyabet süresine göre düzenlemeden sonra aynı korelasyonlar devam etti.

• **Sonuç:** Hs-CRP düzeyleri ile kolestatik karaciğer enzimleri (ALP, GGT) arasındaki pozitif korelasyon, periferik inflamasyon belirteçlerinin fonksiyonel bozukluğu yansıttığını düşündürmektedir. Bununla beraber, hs-CRP düzeyleri, steatoz, inflamasyon veya fibroza ilerlemeyi önceden göstermemektedir.

• **Anahtar Kelimeler:** Non-alkolik steatohepatit, tip 2 diabetes mellitus, yüksek duyarlıklı CRP, Brunt klasifikasyon sistemi. **Nobel Med 2010; 6(2): 43-46**

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is a commonly encountered community health problem which is associated with obesity, hypertriglyceridemia, and insulin resistance.[1] NASH is being increased among patients with type 2 diabetes in recent years. The course of NASH ranges from isolated benign steatosis to a more serious pathological state such as cirrhosis, liver failure or even hepatocellular carcinoma.

Although an isolated steatosis is a benign disease, it may progress to steatohepatitis, cirrhosis, liver failure or even hepatocellular carcinoma in minority of cases. There is no clinical or biochemical indicator predicting the prognosis of NASH.[2] Therefore, it is not possible to predict the course of NASH in which patients may stay as a benign condition or in which it may progress to fibrosis or rarely cirrhosis.

High sensitive C-reactive protein (hs-CRP) is a commonly used acute phase reactant which increases in even mild inflammatory states. It was reported that hs-CRP increased in cases of metabolic syndrome.[3]

In this study, we investigated the relationship between hs-CRP and inflammation in liver tissue in cases with NASH and type 2 diabetes mellitus.

MATERIAL and METHOD

The study included 52 NASH cases with type 2 diabetes mellitus. NASH was diagnosed based on biochemical tests and ultrasound images of the liver. Each patient had a true cut liver biopsy which was guided by ultrasound images, thus the diagnosis was confirmed. Histopathological evaluation of biopsy specimens were performed following Hematoxilen-Eosin and Mason Trichrome stains according to the Brunt Criteria by the same pathologist who was blinded to clinical data. Fasting serum samples were obtained after an overnight fast. Biochemical analyses were done with the appropriate methods; such as fasting blood glucose (FBG), alkaline phosphatase (ALP), aspartat and alanine aminotransferase (AST, and ALT), lactic dehydrogenase (LDH) and gamma glutamyl transpeptidase (GGT) by photometric; hs-CRP by immunoturbidometric and C-peptide by electro-chemiluminescence methods (Roche Diagnostics, Modular System). Insulin resistance was calculated→

using "Homeostasis Model of Assessment" (HOMA-IR) formula (http://www.dtu.ox.ac.uk). C-peptide value was used instead of insulin in patients under insulin therapy. (Statistical Package for the Social Sciences) (SPSS) version 13.0 (2006, SPSS Inc. Chicago, USA), a package program designed for computer, was used for statistical analyses. Pearson test was used for parametric values, and Spearman's rho test was used for non-parametric values in correlation analysis. Parameters not distributed homogenously were log transformed. A value of $p<0.05$ in 95% confidence interval (CI) was considered statistically significant. The study was approved by the Ethical Committee of Istanbul University Medical School and a signed informed consent was obtained from each patient.

RESULTS

The study included 30 (57.7%) male and 22 (42.3%) female cases of NASH with type 2 diabetes mellitus. The mean age between males and females was similar (male; 53.6±7.3 and female; 52.7±7.4 years). Body Mass Index (BMI) in males was lower than females (30.5±3.8 vs. 33.8±3.9 kg/m2 respectively, p= 0.004). The duration of diabetes was also not different between two sexes (7.43±5.20 for males and 7.32±3.98 for females, p>0.05). Table 1 depicted biochemical data of the study participants. All biochemical data were similar between male and female patients, except FBG in females which was significantly higher than males (p= 0.044); in addition, A1c was tended to be higher in females (p= 0.057) (data not shown).

Based on Brunt Criteria, histopathological evaluation of cases is shown in Table 2. Accordingly, majority of our participants was in mild or moderate stages in respect to steatosis, inflammation and fibrosis. Serum levels of hs-CRP were tended to be higher as the severity of steatosis increased, however, did not reach to a statistical difference (3.6±3.4 mg/l in mild, 4.1±2.4 mg/l in moderate, and 6.0±4.4 mg/l in severe groups (Kruskal-Wallis variance analysis p= 0.18). Comparison of participants with and without inflammation (combined grade-1 and grade-2; 4.6±3.0 vs. none; 3.8±3.4 mg/l, p= 0.20) or with and without fibrosis (combined phase-1 and phase-2; 4.4±3.5 vs. none; 3.4±3.0 mg/l, p= 0.29) revealed similar tendency.

Correlation analysis of hs-CRP with clinical and other laboratory parameters is summarized in Table 3. Briefly, hs-CRP was positively correlated with hemoglobin (A1c), FBG, ALP, GGT, and negatively correlated with AST. After adjustment of age and duration of diabetes, the above mentioned correlations remained constantly.

DISCUSSION

Non-alcoholic steatohepatitis has a broad spectrum ranging from a histological simple isolated steatosis to NASH. Although an isolated steatosis has a benign course, steatohepatitis may rarely progress to cirrhosis, hepatic failure or hepatocellular carcinoma.[1] CRP is an acute phase reactant and synthesized by the liver. After developing new techniques to determine high sensitive form, it has been used as a predictor of coronary arterial disease, insulin resistance and type 2 diabetes. Recently, hs-CRP levels were also shown to increase in other low-grade inflammatory disorders such as metabolic syndrome and NASH.[4] Other investigators stated that→

Table 1: Biochemical findings in the study group

	Mean±S.D.	Range (Min-Max)	Median
FBG (mg/dl)	162.9±61.4	81-341	150.5
A1c (%)	7.6±1.7	5.1-12.8	7.0
HOMA-IR	3.0±2.1	0.7-11.4	2.30
TG (mg/dl)	174.7±110.3	32-709	148.0
LDL-C (mg/dl)	108.3±32.9	34-212	105.5
HDL-C (mg/dl)	42.0±9.4	23-74	40.0
ALT (U/l)	64.5±16.3	50-110	57.0
AST (U/l)	40.3±13.6	22-92	38.5
ALP (U/l)	204.3±74.1	89-406	182.5
GGT (U/l)	65.3±70.7	17-460	39.0
C-peptide (ng/ml)	4.1±1.7	1.6-10.0	3.8
hs-CRP (mg/l)	4.0±3.3	0.7-15.0	3.0

Min; minimum, Max; maximum, S.D.; standard deviation, FBG; fasting blood glucose, A1C; hemoglobin A1c, HOMA-IR; Homeostasis model assessment insulin resistance, TG; triglycerides, LDL-C; low density lipoprotein cholesterol, HDL-C; high density lipoprotein cholesterol, ALT; alanine aminotransferase, AST; aspartat aminotransferase, ALP; alkaline phosphatase, GGT; gamma-glutamyl transpeptidase, hs-CRP; high sensitive C-reactive protein. The normal ranges of the parameters are: FBG: 70-100 mg/dl, AST: 5-40 U/L, ALT: 5-40 U/L, LDH: 100-210 U/L, ALP: 30-90 U/L, GGT: 5-60 U/L.

Table 2: Histopathological evaluation of the study group

Steatosis level	n	%
Mild phase (< 33%)	30	57.7
Moderate phase (33-66%)	16	30.8
Severe phase (> 66%)	6	11.5
Inflammatory activity		
Absent	41	78.8
Grade-1	8	15.4
Grade-2	3	5.8
Fibrosis score		
Absent	22	42.3
Phase-1	26	50
Phase-2	4	7.7

Table 3: Correlation analysis of hs-CRP with other laboratory parameters.		
log (hs-CRP) with	**r**	**p**
A1c	0.33	0.017
FBG	0.30	0.032
log (TNF-α)	-0.33	0.018
log (leptin)	0.37	0.009
AST	-0.28	0.048
GGT	0.34	0.014
ALP	0.32	0.020

CRP levels increased during the course of chronic hepatitis and cirrhosis.[5,6]

In this study, we investigated the relationship between hs-CRP levels and severity of histopathological changes in the liver. Our results indicated that hs-CRP levels did not significantly change with any histopathological progress like steatosis, inflammation and fibrosis. Although there was a weak tendency to increase in hs-CRP levels in all three pathological features Koruk et al, reported that they did not find any relationship between CRP levels and the degree of steatosis, inflammation and fibrosis in 18 cases of NASH.[7] However, CRP levels were measured by conventional methods in that study.

In the study by Hui et al, it was stated that hs-CRP levels are not a reliable predictor of the severity of histopathological changes in non-alcoholic fatty liver disease.[4] Also, one study showed that plasma CRP levels are not predictive of the diagnosis of NASH in severely obese patients. Plasma CRP levels were elevated in severely obese patients independently from the presence or absence of Metabolic Syndrome, diabetes or NASH.[8]

Conversely, Uchihara et al. reported that hs-CRP levels were higher in grade-2 and grade 3 cases than grade-1 and isolated steatosis cases. The authors suggested that hs-CRP might be used as a promising biomarker for the screening of NASH.[9] In another study by Iwasaki and et al. found that serum CRP is significantly correlated with HOMA-IR, in both the diabetic patients and the non-diabetic subjects. Although they used computed tomography for liver steatosis, they concluded that serum CRP is related to the degree of liver steatosis in Japanese type 2 diabetes mellitus patients.[10]

We found positive correlations between hs-CRP and variables of glucose control like A1C and FBG in this study. Serum levels of hs-CRP correlated with certain liver enzymes, the correlation was positive with ALP and GGT while AST negatively correlated with hs-CRP. These relationships were maintained when age and duration of diabetes were adjusted. Hui et al, in the above mentioned study, reported that hs-CRP vales positively correlated with age, BMI and HOMA-IR levels.[4] Our study has some limitations. First, the number of participants was somewhat limited. Secondly, the number of cases in the severe histopathological stages was very few. These may mask to possible significant association between any histopathological progress and hs-CRP levels.

CONCLUSION

In conclusion, positive correlations between hs-CRP levels and cholestatic liver enzymes (ALP, GGT) suggested us that peripheral inflammatory markers reflect the functional disorder. However, hs-CRP did not seem to play a predictive role in progressive steatosis, inflammation or fibrosis. In order to find definitive evidence indicating the role of inflammatory process in NASH, large prospectively designed studies should be done in the future.

C **CORRESPONDING AUTHOR:** Gönenç Kocabay MD Dept. of Endocrinology and Metabolism, Fac. of Ist. Med,. Ist. University, Istanbul/TURKEY gonenckocabay@yahoo.com

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