

Serum soluble TWEAK levels are decreased in treatment naive noncirrhotic chronic hepatitis B patients

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Abstract

The mechanisms underlying hepatic inflammation and fibrogenesis in chronic hepatitis B (CHB) are complex and several cytokines are involved. Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a member of the tumor necrosis factor superfamily which also acts as a cytokine. This study was conducted to evaluate serum soluble TWEAK (sTWEAK) levels in noncirrhotic CHB patients.

Fifty-two treatment naive CHB patients and 30 healthy controls were included in the study and serum sTWEAK concentrations were measured using commercially available ELISA kits.

Mean serum sTWEAK concentration was significantly lower in CHB group than healthy controls (189.6 ± 63.3 pg/mL in CHB group and 297.6 ± 61.5 pg/mL in control group, $P < 0.001$). According to the degree of necroinflammation in liver biopsies mean sTWEAK concentrations were found to be 168.14 ± 51.51 , 206.96 ± 58.51 , and 223.62 ± 78.88 pg/mL in patients with mild, moderate, and severe inflammation, respectively, and the difference between groups was statistically significant ($P = 0.022$). sTWEAK concentration was also found to be significantly higher in patients with advanced fibrosis in liver biopsy samples (169.59 ± 52.02 and 211.17 ± 68.22 pg/mL in patients with mild and advanced fibrosis, respectively, $P = 0.016$). Receiver operating characteristic (ROC) curves were obtained in CHB group to differentiate patients with advanced fibrosis from patients with mild fibrosis. Area under curve (AUC) was 0.676 (95% CI; 0.526–0.825) for sTWEAK and for the specified cut-off value of 213.67 pg/mL sensitivity and specificity were 60% and 81.4%, respectively. ROC curve for sTWEAK to differentiate patients with severe inflammation revealed an AUC of 0.664 (95% CI; 0.450–0.878). A cut-off value of 243.27 pg/mL yielded 54.5% sensitivity and 82.9% specificity.

Serum sTWEAK concentration is decreased in treatment naive CHB patients. Further studies with simultaneous determination of circulating sTWEAK concentrations and TWEAK and factor-inducible 14 (Fn14) expressions in the liver biopsy samples would clarify the exact association of TWEAK/Fn14 pathway in the pathogenesis of CHB.

Abbreviations: Alb = Albumin, ALP = Alkaline phosphatase, ALT = Alanine aminotransferase, Anti-HBe = Anti hepatitis B antibody, APRI = AST to Platelet ratio index, AST = Serum aspartate aminotransferase, BMI = Body mass index, CHB = Chronic hepatitis B, CHB = Chronic hepatitis B, D.Bil = Direct bilirubin, FIB-4 = Fibrosis-4 index, Fn14 = Fibroblast growth factor-inducible 14, GGT = Gama-glutamyltransferase, HAI = Histological activity index, HBeAg = Hepatitis B e antigen, HBsAg = Hepatitis B surface antigen, HBV = Hepatitis B virüs, HBV DNA = Hepatitis B virus DNA, HCV = Hepatitis C virüs, HDL = High density lipoprotein, IL-1b = Interleukin-1b, IL-6 = Interleukin 6, LDL-C = low density lipoprotein, PLT = Platelet count, ROC = Receiver operating characteristic, SD = Standard deviation, sTWEAK = Soluble tumor necrosis factor-like weak inducer of apoptosis, T.Bil = Total bilirubin, TG = triglyceride, TGF β = Transforming growth factor β , TNF α = Tumor necrosis factor alpha, TWEAK = Tumor necrosis factor-like weak inducer of apoptosis.

Keywords: chronic hepatitis B, fibrosis, inflammation, sTWEAK, tumor necrosis factor-like weak inducer of apoptosis

1. Introduction

Chronic hepatitis B virus (HBV) infection is a global health problem. It causes a wide spectrum of liver diseases including

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acute hepatitis B, inactive hepatitis B carrier state, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. HBV prevalence shows wide variations among various regions of the world with an estimated global prevalence of 3.6%.^[1] Chronic hepatitis B (CHB) is associated with various degrees of liver inflammation and fibrosis and may progress to cirrhosis. The mechanisms underlying hepatic inflammation and fibrogenesis are complex involving interaction of hepatocytes, nonparenchymal liver cells, and inflammatory cells. Although the exact pathophysiological mechanisms underlying hepatic inflammation and fibrosis are not fully understood several cytokines such as tumor necrosis factor alpha (TNF) α , interleukin (IL)-1b and IL-6, and transforming growth factor (TGF) β are shown to take role.^[2] Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a cell surface-associated type II transmembrane protein which is a member of the TNF superfamily. Soluble TWEAK (sTWEAK) is generated by proteolytical processing of membrane bound TWEAK and it functions as a cytokine.^[3] sTWEAK exerts its biological effects through binding to its receptor known as fibroblast growth factor-inducible 14 (Fn14).

TWEAK/Fn14 axis has been shown to be associated with the development of tissue fibrosis in colon, heart, kidney, and skeletal muscle in several chronic diseases and experimental models.^[4-7] TWEAK was also been implicated in the pathogenesis of liver inflammation and fibrosis.^[8-12] Therefore this study was conducted to evaluate serum sTWEAK levels in patients with treatment naive noncirrhotic chronic hepatitis B patients.

2. Materials and methods

This cross-sectional study included 52 patients with CHB and 30 healthy controls. The study protocol was approved by the local Comity of Ethics of Necmettin Erbakan University, Meram Faculty of Medicine. Written informed consents were taken from the participants in both study and control groups. CHB group included patients who were hepatitis B surface antigen (HBsAg) positive for at least 6 months with active viral replication (HBV DNA > 2000 IU/mL) and various degrees of inflammation and fibrosis confirmed with liver biopsy samples. Anti-HCV antibody, antinuclear antibody, antismooth muscle antibody, antiliver-kidney microsomal antibody, serum copper and ceruloplasmin levels and serum transferrin saturation were obtained for all patients in the CHB group to exclude any other cause of chronic liver disease. Liver biopsies were performed for every person in CHB group. Ishak et al^[13] scoring system was used for grading necroinflammation and fibrosis in liver biopsy samples. In terms of necroinflammation CHB patients were divided into 3 groups according to histological activity indexes (HAI): mild inflammation (HAI: 1–8), moderate inflammation (HAI: 9–12), severe inflammation (HAI: 13–18) and according to the degree of fibrosis in liver biopsy samples, patients were categorized in 2 groups: mild fibrosis (Ishak stages: 1 and 2), advanced fibrosis (Ishak stages: 3 and 4). Since the study design included noncirrhotic patients only, patients with Ishak 5 to 6 fibrosis were excluded. Blood samples for biochemical analysis and sTWEAK were obtained early in the morning on the day of liver biopsy. Control group consisted of 30 healthy people with normal liver enzymes and negative serological tests for hepatitis B and C (hepatitis B surface antigen, hepatitis B core antigen, anti-HCV antibody). Exclusion criteria were as follows: presence of cirrhosis (patients with clinical, laboratory, and radiologic signs of cirrhosis or patients with modified Ishak stage 5 and 6 fibrosis in liver biopsy samples), patients with chronic liver diseases of any other etiology or considerable alcohol consumption (>20 g/day for males and >10 g/day for females), patients with known malignancies, chronic renal diseases, autoimmune diseases, or any ongoing active infections. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT) activities as well as other biochemical parameters were measured by using standard autoanalyzer methods on Abbott Architect 16000 system with the original reagents according to the manufacturer's instructions (Abbott Laboratories, Abbott Park, IL). Serum samples for sTWEAK were separated by centrifugation at 4000 rpm/min for 5 minutes at 4°C and were immediately transferred to freezer to be stored at –80°C until they were analyzed. sTWEAK levels were measured with a commercially available kit based on enzyme-linked immunosorbent assay (eBioscience, San Diego, CA, USA, Human TWEAK Instant Elisa, Cat no: BMS2006INST). The results were expressed as pg/mL. As noninvasive scores of fibrosis, AST to Platelet Ratio Index (APRI), and Fibrosis-4

(FIB-4) index were calculated in both control and CHB groups using formulas:

$$\text{APRI} = \left[\frac{\text{AST (U/L)}}{\text{AST (U/L) upper limit normal}} \right] / \text{PLT} (10^9/\text{L}) \times 100 \text{ and FIB-4} = \text{age (years)} \times \text{AST (U/L)} / \left[\text{Plt} (10^9/\text{L}) \times (\text{ALT (U/L)})^{1/2} \right].$$

Statistical analyses were done using computer software “Statistical Package for Social Sciences V.19.0” for Windows. Continuous variables were expressed as mean ± standard deviation. Continuous variables were tested for normal distribution using 1 sample Kolmogorov–Smirnov test. Comparisons were done using Student *t* test (2 group comparison) and 1-way Anova test (comparison of more than 2 groups) when the tested parameter was normally distributed. On the other hand Mann–Whitney *U* test was used to search for the significance of difference between 2 groups and Kruskal–Wallis test for more than 2 groups when the tested variable was not normally distributed. The significance of the linear correlation between continuous variables was tested using the Spearman correlation test. Receiver operating characteristic (ROC) curves were obtained for sTWEAK to predict severe inflammation and advanced fibrosis. Sensitivity, specificity, positive and negative predictive values were calculated for specified cutoff values. ROC curves were also obtained for APRI and FIB-4 to predict advanced fibrosis. Statistical significance for all analyses were defined as $P < 0.05$.

3. Results

There were 32 males (61.5%) and 20 females (38.5%) in CHB group with a mean age of 41.22 ± 11.50 years and 17 males (56.7%) and 13 females (43.3%) in the control group with a mean age of 41.22 ± 11.50 years. Age and gender distribution were found to be similar in both groups. All patients in CHB group were HBeAg negative and anti-HBe positive. Demographical and laboratory data of the groups are summarized in Table 1.

Mean serum sTWEAK concentration in CHB group was 189.6 ± 63.3 pg/mL and it was significantly lower than healthy control group which had a mean serum sTWEAK concentration of 297.6 ± 61.5 pg/mL ($P < 0.001$) (Fig. 1).

3.1. sTWEAK and liver inflammation

According to the degree of necroinflammation in liver biopsies CHB patients were divided into 3 groups as previously described. There were 28 patients (53.8%) with mild, 13 patients (25.0%) with moderate, and 11 patients (21.2%) with severe inflammation. Mean sTWEAK concentrations were 168.14 ± 51.51 , 206.96 ± 58.51 , and 223.62 ± 78.88 pg/mL in patients with mild, moderate, and severe inflammation, respectively, and the difference between the groups were statistically significant ($P = 0.022$). Statistical analyses revealed that all 3 groups had significantly lower serum sTWEAK levels than healthy controls ($P < 0.001$, $P < 0.001$, and $P = 0.003$, respectively) and both moderate and severe inflammation group had significantly higher serum sTWEAK levels than patients with mild inflammation ($P = 0.038$ and $P = 0.014$, respectively). On the other hand the difference between moderate and severe inflammation groups was not statistically significant ($P = 0.559$). Mean serum sTWEAK concentrations in patients with mild, moderate, and severe inflammation are illustrated in Fig. 2.

Table 1
Demographical and laboratory data of the groups.

	Chronic hepatitis B group, mean ± SD (range)	Healthy controls, mean ± SD (range)	P
Age, y*	43.98 ± 11.73 (21–69)	43.6 ± 8.84 (25–61)	0.878
Male (n), %	32/61.5	17/56.7	0.665
sTWEAK, pg/mL*	189.58 ± 63.32 (62.60–327.25)	297.60 ± 61.48 (192.60–409.41)	<0.0001
FIB-4†	1.21 ± 0.88 (0.09–4.16)	0.84 ± 0.28 (0.31–1.46)	0.016
APRI†	0.46 ± 0.36 (0.05–1.71)	0.23 ± 0.07 (0.15–0.39)	<0.0001
BMI, kg/m ² *	26.811 ± 4.18 (17.51–40.23)	24.761 ± 3.17 (19.29–32.65)	0.021
Glucose, mg/dL*	101.88 ± 30.36 (69–206)	98.97 ± 12.73 (74–138)	0.577
Creatinine, mg/dL*	0.76 ± 0.14 (0.45–1.16)	0.77 ± 0.15 (0.56–1.02)	0.729
AST, U/L*	50.12 ± 65.18 (7–382)	21.40 ± 6.40 (13–35)	0.003
ALT, U/L*	36.44 ± 31.36 (14–169)	20.93 ± 12.63 (7–35)	0.003
GGT, U/L*	34.93 ± 31.34 (10–169)	27.88 ± 14.23 (10–63)	0.731
ALP, U/L*	84.65 ± 31.46 (48–210)	69.71 ± 14.10 (46–108)	0.012
T.Bil., mg/dL*	0.94 ± 0.57 (0.28–3.64)	0.65 ± 0.36 (0.32–1.15)	0.004
D.Bil., mg/dL*	0.32 ± 0.26 (0.1–2)a	0.20 ± 0.09 (0.1–0.49)	0.001
Albumin, g/dL*	4.27 ± 0.39 (3.1–4.8)	4.33 ± 0.38 (3.40–4.90)	0.543
Total cholesterol, mg/dL*	183.19 ± 45.77 (108–305)	205.46 ± 43.78 (144–277)	0.152
LDL-C, mg/dL*	124.70 ± 37.72 (69–219)	120.91 ± 46.43 (40–184)	0.795
HDL-C, mg/dL*	41.70 ± 10.84 (26–63)	48.94 ± 13.54 (36–83)	0.166
TG, mg/dL*	112.38 ± 39.98 (47–184)	117.70 ± 49.93 (38–179)	0.725
Leukocyte, mm ³ *	6.98 ± 2.04 (3.90–9.80)	7.33 ± 1.24 (4.9–9.70)	0.285
Neutrophil, mm ³ *	4.01 ± 1.53 (1.89–7.20)	4.17 ± 0.98 (2.2–6.40)	0.598
Lymphocytes, mm ³ *	2.30 ± 0.75 (1.20–4.79)	2.47 ± 0.68 (1.20–4.00)	0.322
Hemoglobin, g/dL*	14.12 ± 2.05 (8.5–17.10)	14.86 ± 1.38 (11.7–17.2)	0.054
Platelet count, mm ³ *	255.12 ± 172.67 (103–1338)	263.23 ± 47.50 (160–354)	0.802

Alb = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, APRI = AST to Platelet Ratio Index, AST = aspartate aminotransferase, BMI = body mass index, D.Bil = direct bilirubin, FIB-4 = fibrosis-4 score, GGT = gamma-glutamyltransferase, HBV DNA = hepatitis B virus DNA, HDL = high density lipoprotein, LDL-C = low density lipoprotein, SD = standard deviation, sTWEAK = soluble tumor necrosis factor-like weak inducer of apoptosis, T.Bil = total bilirubin, TG = triglyceride.

Bold values were used to emphasize for the statistical significance between groups.

*Parameter is normally distributed, Student *t* test was used.

†Parameter is not normally distributed, Mann-Whitney *U* test was used.

ROC curves were obtained for sTWEAK, AST, and ALT to differentiate patients with severe inflammation from patients with mild and moderate inflammation. Computed AUC were 0.664 (95% CI; 0.450–0.878) for sTWEAK, 0.630 (95% CI; 0.441–0.819) for AST, and 0.561 (95% CI; 0.349–0.774) for ALT. For sTWEAK, specified cut-off value of 243.27 pg/mL yielded 54.5% sensitivity and 82.9% specificity and calculated positive and negative predictive values were 46.2% and 87.2%,

respectively. ROC curves for sTWEAK, AST, and ALT to differentiate patients with severe inflammation are summarized in Fig. 3.

3.2. sTWEAK and liver fibrosis

In terms of fibrosis stage in liver biopsy samples, CHB patients were divided into 2 groups as previously described. There were

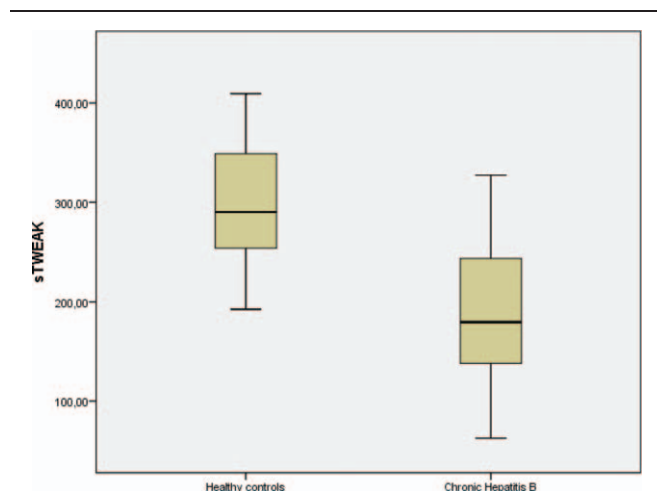


Figure 1. Mean serum sTWEAK concentrations in chronic hepatitis B group and healthy controls.

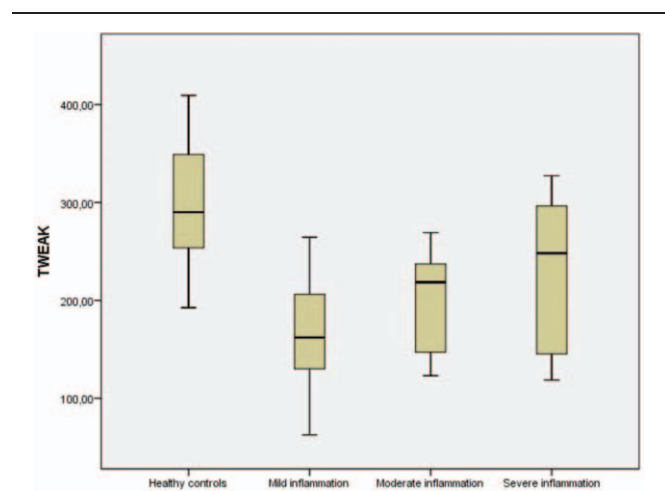


Figure 2. Mean serum sTWEAK concentrations in patients with mild, moderate, and severe inflammation in liver biopsies.

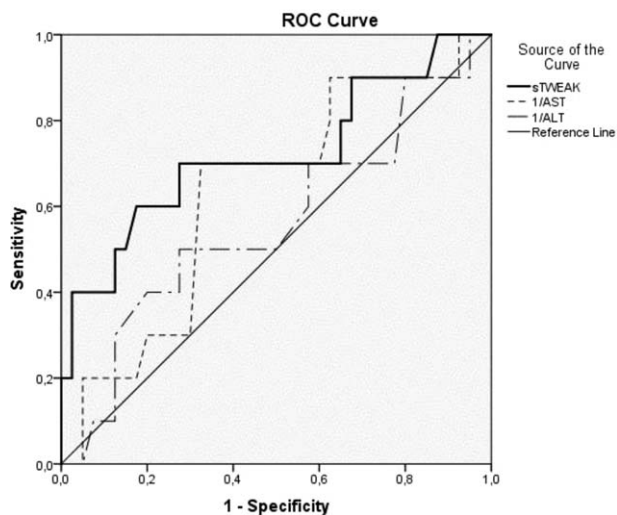


Figure 3. ROC curves for sTWEAK, AST, and ALT to differentiate chronic hepatitis B patients with severe inflammation.

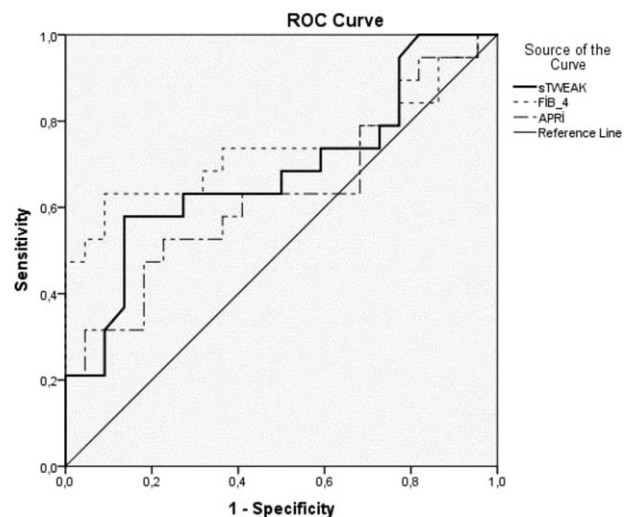


Figure 5. ROC curves for sTWEAK, APRI, and FIB4 scores to differentiate chronic hepatitis B patients with advanced fibrosis.

27 patients (51.9%) with mild fibrosis and 25 patients (48.1%) with advanced fibrosis. Mean sTWEAK concentrations were 169.59 ± 52.02 and 211.17 ± 68.22 pg/mL in mild and advanced fibrosis groups, respectively, and both groups were found to have significantly lower serum sTWEAK concentrations than healthy controls ($P < 0.001$ for both comparisons). The difference between mild fibrosis and advanced fibrosis groups was also found to be statistically significant ($P = 0.016$). Mean serum sTWEAK concentrations in mild and advanced fibrosis groups are illustrated in Fig. 4.

APRI score and FIB-4 indexes were computed in CHB and control groups. As it would be expected mean APRI score and mean FIB-4 index were found to be significantly higher in CHB group than healthy controls (0.46 ± 0.36 and 0.23 ± 0.07 for APRI and 1.21 ± 0.88 and 0.84 ± 0.28 for FIB-4 for CHB and control groups, respectively). Mean FIB-4 score was 1.64 ± 1.09 in patients with advanced fibrosis and it was significantly higher than patients with mild fibrosis which had a mean FIB-4 score of 0.81 ± 0.32 ($P < 0.001$). APRI score was also found to be

significantly higher in patients with advanced fibrosis (0.60 ± 0.44 and 0.32 ± 0.18 in advanced fibrosis and mild fibrosis group, respectively, $P < 0.001$).

ROC curves were obtained in CHB group for APRI, FIB-4, and sTWEAK to differentiate patients with advanced fibrosis from patients with mild fibrosis. Computed area under curves (AUC) were 0.676 (95% CI; 0.526–0.825) for sTWEAK, 0.674 (95% CI; 0.511–0.836) for APRI and 0.748 (95% CI; 0.585–0.910) for FIB-4 index. For sTWEAK, specified cut-off value of 213.67 pg/mL revealed a sensitivity of 60% and a specificity of 81.4% and calculated positive and negative predictive values were 75% and 68.7%, respectively. ROC curves for sTWEAK, APRI, and FIB-4 to differentiate patients with advanced fibrosis are summarized in Fig. 5.

3.3. Correlation analysis

Correlation analysis revealed that serum sTWEAK concentration was positively correlated with the histological activity index ($r(48) = 0.339$, $P = 0.014$) and fibrosis stage ($r(48) = 0.330$, $P = 0.017$) and negatively correlated with platelet count ($r(48) = -0.317$, $P = 0.022$) in CHB patients but all reported correlations were weak. Multivariate linear regression analyses for age, sex, histological activity index, and fibrosis stage showed that only histological activity index was independently associated with serum sTWEAK levels ($R^2 = 0.115$, $P = 0.014$, $\beta = 0.339$). Correlation analyses are summarized in Table 2.

4. Discussion

CHB is still one of the major causes of cirrhosis and hepatocellular cancer worldwide. Necroinflammation and fibrosis in liver are the major determinant of the clinical outcome and long-term consequences in CHB patients. The ultimate goal of CHB treatment is total viral elimination and normalization of liver histology. Unfortunately currently used oral antiviral drugs suppress viral replication with resultant clinical, biochemical, and histological improvements but total viral elimination and cure is not possible. Liver fibrosis associated with CHB also regresses to some extent with the use of oral antiviral drugs but there is still an

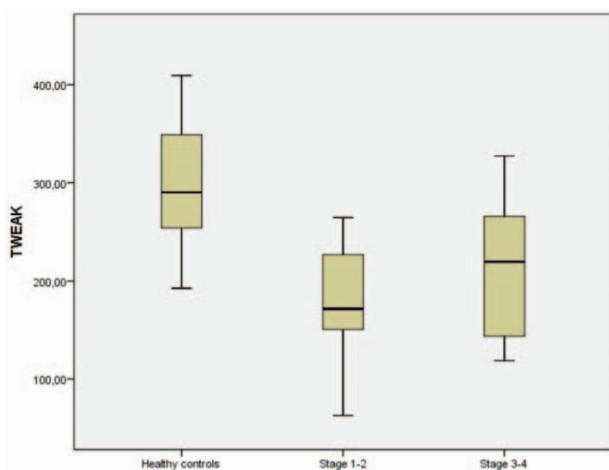


Figure 4. Mean serum sTWEAK concentrations in patients with mild and advanced fibrosis in liver biopsies.

Table 2**Correlation analysis of sTWEAK with various demographical, clinical, and laboratory parameters.**

	P	Correlation coefficient
Age, y	0.190	0.146*
HBV DNA, copy/mL	0.295	-0.093†
Fibrosis stage (Stage 1–4)	0.017	0.330†
HAI (1–18)	0.014	0.339*
Glucose, mg/dL	0.702	0.046*
AST, U/L	0.421	-0.091*
ALT, U/L	0.459	-0.084*
Albumin, g/dL	0.973	-0.004*
Platelet count, mm ³	0.022	-0.317*

ALT = alanine aminotransferase, AST = aspartate aminotransferase, HAI = histologic activity index, HBV DNA = hepatitis B virus DNA, sTWEAK = serum soluble tumor necrosis factor-like weak inducer of apoptosis.

Bold values were used to emphasize for the statistical significance between groups.

* Pearson correlation coefficient (*r*).

† Spearman correlation coefficient (*rho*).

obvious need for new drugs that provides regression of fibrosis and improvement of liver histology. Therefore identification of the exact pathophysiologic mechanisms underlying liver fibrosis and inflammation would expose new targets for the development of antifibrotic drugs.

As previously noted, TWEAK/Fn14 axis has been shown to be associated with the development of tissue fibrosis in colon, heart, kidney, and skeletal muscle in several studies. Actually, there are also several studies in the literature implicating the possible role of sTWEAK in the pathogenesis of inflammation and fibrosis associated with various acute and chronic forms of liver injury.^[14,15] Liver progenitor cells are the key cells in liver regeneration and TWEAK/Fn14 axis has also been shown to stimulate liver progenitor cells, hepatocytes, and cholangiocytes.^[12] But to our knowledge, there is no study in the literature investigating TWEAK/Fn14 pathway in CHB patients.

The results of this study have shown that serum sTWEAK levels were lower in CHB patients than healthy controls. Since there is no other study in the literature investigating serum sTWEAK concentration in CHB patients, it is hard to interpret our results and compare it with the literature. At first glance, the results of this study seems to be contradictory because assuming that TWEAK is involved in the pathogenesis of liver inflammation and fibrosis, it would be expected to increase in CHB patients. But we believe that the exact setting in vivo may be much more complex. A possible explanation would be dramatic upregulation of Fn14 expression and Fn14 receptors within the liver due to ongoing tissue injury and regeneration in CHB patients. In this setting, Fn14 receptors will bind circulating sTWEAK and cascades of inflammation and fibrosis are activated within the liver, but at the same time serum-free sTWEAK concentration is reduced due to entrapment by increased Fn14 receptors. In favor of this hypothesis, Fn14 has been shown to be very inducible in several studies. TWEAK and Fn14 are expressed in very low quantities in healthy livers but undergoes dramatic upregulation in case of tissue injury. In a study conducted by Wilhelm et al,^[8] it has been shown that expression of TWEAK and Fn14 were very low in normal liver tissue samples whereas Fn14 expression was increased up to 58-fold after carbon tetrachloride induced liver injury. In another study it has been shown that baseline Fn14 expression was very low in liver samples from healthy mice and rapidly undergoes approximately 50 times increase after partial hepatectomy.^[16] Moreover, Affò

et al^[9] have reported that several TNF superfamily receptors but not their ligands were markedly overexpressed in livers with alcoholic hepatitis. In that study, Fn14 expression was reported to increase up to 20-fold. The results of all these studies suggest that Fn14 is dramatically increased after various forms of hepatocellular injury. To our knowledge there is no study in the literature investigating expression of TWEAK/Fn14 in the liver biopsies of CHB patients but since there is ongoing active chronic tissue injury TWEAK/Fn14 is also expected to increase in CHB patients through similar mechanisms. As stated earlier such a local increase in Fn14 would result in entrapment of circulating sTWEAK molecules by different cell types overexpressing Fn14 receptors within the liver. There would be 2 possible consequences associated with this setting; first circulating free sTWEAK concentration would be decreased which is consistent with our results, secondly increased sTWEAK/Fn14 interaction would stimulate inflammatory and fibrogenetic pathways within the liver. Another finding supporting our hypothesis is that sTWEAK concentrations were also shown to decrease in other chronic liver diseases as well. In parallel to our results, Lozano-Bartolomé et al^[10] have reported that serum sTWEAK levels were lower in patients with fatty liver disease and nonalcoholic steatohepatitis than healthy controls. Similarly, in another study, we have also found that serum sTWEAK concentration was lower in patients with biopsy proven nonalcoholic steatohepatitis than patients with simple hepatosteatosis and healthy controls (Asil M, MD, unpublished data, June 2016). Since cell types and humoral mechanisms mediating inflammation and fibrogenesis in liver are similar in various chronic liver diseases we believe that similar mechanisms may be responsible from this low sTWEAK concentrations in patients with nonalcoholic steatohepatitis.

Another point to emphasize is that the results of this study have shown that serum sTWEAK concentration was associated with the degree of inflammation and fibrosis in liver biopsy samples. sTWEAK concentration was significantly higher in patients with moderate and severe inflammation than patients with mild inflammation and patients with advanced fibrosis had significantly higher serum sTWEAK concentration than patients with mild fibrosis. Correlation analyses showed that serum sTWEAK concentration was correlated with histological activity index and fibrosis stage but correlations were weak. ROC analyses also revealed low sensitivity and moderate specificity for both inflammation and fibrosis. Therefore, use of sTWEAK solely as a marker of either liver fibrosis or inflammation seems to be unwarranted. But taking into account that there is no ideal marker for the prediction of liver inflammation and fibrosis we believe that sTWEAK may have a potential use to be included in different marker panels.

The main limitation of this study is its cross-sectional design therefore it is hard to establish a causality relationship between TWEAK and hepatocellular inflammation and fibrosis. Further studies with simultaneous determination of serum sTWEAK concentrations and TWEAK and Fn14 expressions in the liver biopsy samples would clarify the possible role of TWEAK/Fn14 pathway in the pathogenesis of CHB. Moreover, there may be some other factors such circulating antibodies to sTWEAK that may possibly affect sTWEAK concentrations in CHB patients and the study design does not make it possible to determine such factors.

In conclusion, serum sTWEAK concentration is decreased in patients with CHB. sTWEAK may have a potential use to be included in marker panels predicting inflammation and fibrosis in

CHB patients. Further studies are needed to clarify the exact role of TWEAK/Fn14 pathway in the pathogenesis of CHB.

References

- [1] Schweitzer A, Horn J, Mikolajczyk RT, et al. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet* 2015;386:1546–55.
- [2] Trautwein C, Friedman SL, Schuppan D, et al. Hepatic fibrosis: concept to treatment. *J Hepatol* 2015;62(1 suppl):S15–24.
- [3] Burkly LC, Michaelson JS, Hahm K, et al. TWEAKing tissue remodeling by a multifunctional cytokine: role of TWEAK/Fn14 pathway in health and disease. *Cytokine* 2007;40:1–6.
- [4] Son A, Oshio T, Kawamura YI, et al. TWEAK/Fn14 pathway promotes a T helper 2-type chronic colitis with fibrosis in mice. *Mucosal Immunol* 2013;6:1131–42.
- [5] Chen HN, Wang DJ, Ren MY, et al. TWEAK/Fn14 promotes the proliferation and collagen synthesis of rat cardiac fibroblasts via the NF- κ B pathway. *Mol Biol Rep* 2012;39:8231–41.
- [6] Uvero AC, Benito-Martin A, Fuentes-Calvo I, et al. TNF-related weak inducer of apoptosis (TWEAK) promotes kidney fibrosis and Ras-dependent proliferation of cultured renal fibroblast. *Biochim Biophys Acta* 2013;1832:1744–55.
- [7] Yarar-Fisher C, Bickel CS, Kelly NA, et al. Heightened TWEAK-NF- κ B signaling and inflammation-associated fibrosis in paralyzed muscles of men with chronic spinal cord injury. *Am J Physiol Endocrinol Metab* 2016;310:E754–61.
- [8] Wilhelm A, Shepherd EL, Amatucci A, et al. Interaction of TWEAK with Fn14 leads to the progression of fibrotic liver disease by directly modulating hepatic stellate cell proliferation. *J Pathol* 2016; 239:109–21.
- [9] Affò S, Dominguez M, Lozano JJ, et al. Transcriptome analysis identifies TNF superfamily receptors as potential therapeutic targets in alcoholic hepatitis. *Gut* 2013;62:452–60.
- [10] Lozano-Bartolomé J, Llauradó G, Rodríguez MM, et al. Reduced circulating levels of sTWEAK are associated with NAFLD and may affect hepatocyte triglyceride accumulation. *Int J Obes (Lond)* 2016;doi: 10.1038/ijo.2016.73. [Epub ahead of print].
- [11] Karaca G, Xie G, Moylan C, et al. Role of Fn14 in acute alcoholic steatohepatitis in mice. *Am J Physiol Gastrointest Liver Physiol* 2015; 308:G325–34.
- [12] Tirnitz-Parker JE, Viebahn CS, Jakubowski A, et al. Tumor necrosis factor-like weak inducer of apoptosis is a mitogen for liver progenitor cells. *Hepatology* 2010;52:291–302.
- [13] Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696–9.
- [14] Feng SLY, Guo Y, Factor VM, et al. The Fn14 immediate-early response gene is induced during liver regeneration and highly expressed in both human and murine hepatocellular carcinomas. *Am J Pathol* 2000;156: 1253–61.
- [15] Jakubowski A, Ambrose C, Parr M, et al. TWEAK induces liver progenitor cell proliferation. *J Clin Invest* 2005;115:2330–40.
- [16] Karaca G, Swiderska-Syn M, Xie G, et al. TWEAK/Fn14 signaling is required for liver regeneration after partial hepatectomy in mice. *PLoS ONE* 2014;9:e83987.