SCIE DOI: 10.14744/scie.2024.00187
South. Clin. Ist. Euras. 2024;35(2):109-113

Original Article

The Clinical Significance of Fibrinogen-Like Protein 2 (FGL-2) Levels in Nonalcoholic Steatohepatitis

Deniz Işık, Heves Sürmeli

Department of İnternal Medicine, Kartal Dr Lütfi Kırdar City Hospital, Division of Oncology, İstanbul, Türkiye

Submitted: 30.11.2023
Revised: 24.05.2024
Accepted: 22.05.2024

Correspondence: Deniz Işık, Kartal Dr Lütfi Kırdar City Hospital, İstanbul, Türkiye
E-mail: dnz.1984@yahoo.com

Keywords: FGL-2 levels; nonalcoholic steatohepatitis.

ABSTRACT

Objective: Non-alcoholic steatohepatitis (NASH) is one of the most common metabolic diseases of the liver. In patients with NASH, progression to cirrhosis can be observed in approximately 25% over a decade following diagnosis, and it is considered the most frequent cause of cryptogenic cirrhosis. Fibrinogen-like protein-2 (FGL-2) is a sub-member of the fibrinogen superfamily secreted by regulatory T cells. It inhibits the maturation of dendritic cells and induces apoptosis in B cells, playing a role in innate and adaptive immunity regulation. Recent studies have shown high serum FGL-2 levels are a poor prognostic indicator in patients with viral hepatitis.

Methods: The study included 30 NASH patients diagnosed by biopsy and a control group of 21 healthy individuals. Serum FGL-2 levels of individuals in both groups were compared. The relationship between the clinicopathological features of individuals in both groups and FGL-2 levels was also examined.

Results: Serum FGL-2 levels in NASH patients were found to be 47±12 ng/ml, while in the control group, it was 37±10 ng/ml. Statistical evaluation was performed using the Mann-Whitney U test. A significant difference was found between patients classified by biopsy as simple steatosis, borderline NASH, definite NASH, and the control group (p=0.002). A significant difference was also observed when evaluating these four groups among themselves (Kruskal-Wallis test, p=0.012).

Conclusion: Our study identified higher serum FGL-2 levels in patients diagnosed with NASH, consistent with steatohepatitis observed in biopsies. Elevated FGL-2 levels may indicate hepatic damage at the microinflammation level. Estimating disease severity, non-invasive monitoring and control of disease progression, selecting patients for aggressive treatment approaches, and intensifying monitoring intervals for cirrhosis and HCC could be crucial. However, more comprehensive studies on this topic are needed.

INTRODUCTION

Fatty liver disease is categorized into alcoholic and non-alcoholic fatty liver disease (NAFLD). NAFLD is a broad spectrum of disorders characterized by predominant macrovesicular steatosis in individuals without significant alcohol consumption.[1-4] NAFLD encompasses two distinct conditions: simple fatty liver without inflammation and fibrosis and non-alcoholic steatohepatitis (NASH) with steatosis and necroinflammatory activity.[5] Simple fatty liver disease presents a benign clinical course without chronic hepatitis or progression to fibrosis. In contrast, many NASH patients exhibit established chronic liver damage and even cirrhosis at diagnosis.[4-7] Liver biopsy remains the sole method for diagnostic differentiation between the two.[2,6] However, liver biopsy is invasive, may require hospitalization, can be painful, carries a risk of complications, is costly, and can be burdensome for both the patient and physician due to its potential for false negatives and the need for repeated procedures.

Fibrinogen-like protein 2 (FGL-2), also known as fibroleukin, was initially cloned from cytotoxic T lymphocytes. Due to its 36% similarity with fibrinogen beta and alpha chains, it is classified as a member of the fibrinogen superfamily.[8] FGL-2 primarily exists as a membrane-associated protein on the surface of macrophages and endothelial cells. This protein possesses prothrombinase activity, facilitating the conversion of prothrombin to thrombin.[9] Secreted by Treg cells, FGL-2 inhibits dendritic cell (DC) maturation and function and induces B cell apoptosis, thereby modulating immunoregulatory effects

[10-13] Animal studies have shown that serum FGL-2 levels increase in viral hepatitis and correlate with liver damage. [14,15] Reports suggest a relationship between serum FGL-2 levels and the extent and fibrosis of chronic hepatitis C.[16] In this study, we aimed to determine the predictive value of fibrinogen-like protein 2 (FGL-2) levels in forecasting the degree of hepatosteatitis in NAFLD.

MATERIALS AND METHODS

Patient Population

Our study included 30 patients monitored for Non-Alcoholic Fatty Liver Disease (NAFLD) and a healthy control group of 21 individuals. Inclusion criteria for the patients were: 1- No history of alcohol consumption (>20 mg/day) or substance addiction. 2- Negative ELISA results for HBsAg, anti-HCV, and anti-HIV. 3- Absence of conditions like hemochromatosis, Wilson's disease, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, biliary obstruction, alpha-1 antitrypsin deficiency, and malignancy. 4- Absence of thrombotic diseases and non-use of anticoagulants.

Liver biopsies were performed on NAFLD patients. Concurrently, hematological, biochemical, waist, and hip circumference measurements were recorded. Patients were staged post-liver biopsy according to the Kleiner classification. Steatosis was semi-quantitatively scored based on its degree: <%5=0, %5-33=1, %33-66=2, >66=3. Lobular inflammation was scored as Stage 0: no affected lobules, Stage 1: <2 foci/x 200 magnification, Stage 2: 2-4 foci/x200 magnification, Stage 3: >4 foci/x200 magnification. Fibrosis was graded as follows: Stage 1: perisinusoidal or periportal, 1A: mild, zone 3, 1B: moderate, zone 3 and perisinusoidal fibrosis, 1C: portal and periportal fibrosis, Stage 2: perisinusoidal, portal, and periportal fibrosis, Stage 3: bridging fibrosis, Stage 4: Cirrhosis. NASH scores were obtained by summing the values of steatosis (0-3), lobular inflammation (0-3), and ballooning degeneration (0-2), resulting in scores ranging from 0-8. Scores of 0-2 were considered simple steatosis, 3-4 as borderline NASH, and > four as definite NASH.

The study was conducted in accordance with the Helsinki Declaration. All patients were informed about the study and provided written consent.

Statistical Analysis

Depending on the data distribution, the Mann-Whitney U and Independent Samples t-test were used to compare two independent groups. Distribution and variance analyses were performed for independent groups with more than two categories. ANOVA was used for groups with normal distribution and variances, and the Kruskal-Wallis test for those without. The correlation between continuous variables was calculated using the Pearson correlation coefficient. The Pearson Chi-Square and Fisher's exact tests were employed for categorical variables. The predictive value of FGL-2 levels in identifying NAFLD patients was assessed using ROC analysis. Means and standard deviations are provided. A significance level of $P<0.05$ was adopted. Statistical calculations were performed using SPSS 17.0 software.

RESULTS

The clinical and laboratory characteristics of the cases in our study are described in Table 1. There were no significant differences between the groups regarding age, gender, Hg, Htc, and Plt values. However, significant differences were observed for AST, ALT, HOMA IR, BMI, waist circumference, and serum FGL-2 levels. ($p<0.001$, <0.001, =0.001, =0.003, =0.0035, and =0.012, respectively). The histopathological features of the patients who underwent liver biopsy are presented in Table 2.

Serum FGL-2 levels were found to be 47±12 ng/ml in patients with biopsy-proven NAFLD, compared to 37±10 ng/ml in the control group. Upon evaluation using the Mann-Whitney U test, a significant difference was observed between the groups. (p=0.002, Figure 1) Comparisons within

Table 1. Demographic and characteristic features of the patients

Groups	Simple steatozis (n=7)	Borderline NASH (n=11)	NASH (n=12)	Control (n=21)	p
Age	47±14	45±15	45±12	37±10	NS
Gender (F/M)	5/2	6/5	5/7	13/8	NS
Hgb	14.3±1.4	14.1±1.6	13.7±1.2	13.9±1.3	NS
WBC	7787±2247	7654±3152	7670±1842	7392±1649	NS
Plt (x10^3)	236±63	258± 48	269±111	279±48	NS
AST	48±27	43±18	40±15	21±7	<0.001
ALT	62±42	52±24	46±17	25±13	<0.001
HOMA-IR	2.9±1.8	2.6±1.8	3.2±2.0	0.95±0.53	0.001
BMI	30±3	32±12	29±17	24±3	0.003
Waist circumference (cm)	99±22	99±12	104±17	86±12	0.035
FGL-2 (ng/ml)	43.6±12.3	46.1±9.7	51.2±13.5	37.6±19.3	0.012

Table 2. Histopathological findings in NAFLD patients

Steatoz	**n (%)**
0	1 (3.3)
1	15 (50)
2	9 (30)
3	5 (16.7)
NASH score	
1	1 (3.3)
2	4 (13.3)
3	2 (6.7)
4	6 (20)
5	5 (16.7)
6	5 (16.7)
7	6 (20)
8	1 (3.3)
Fibrozis Stage	
0	4 (13.3)
1	18 (60)
2	5 (16.7)
3	2 (6.6)
4	1 (3.3)

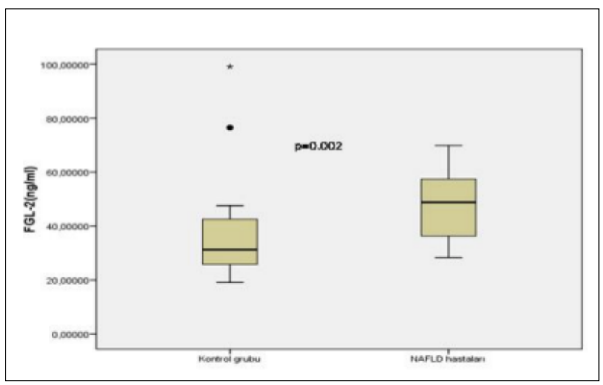

Figure 1. Mean FGL-2 (ng/ml) levels in NAFLD patients and the Control group.

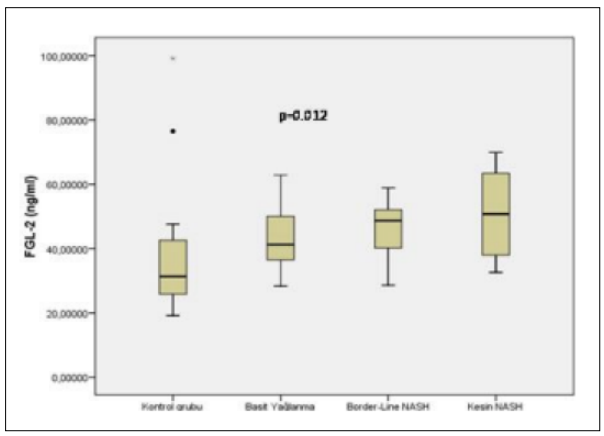

Figure 2. Mean FGL-2 (ng/ml) levels in subgroups of NAFLD patients and the Control group.

the four groups using the Kruskal-Wallis test also revealed significant differences. (p=0.012, Figure 2)

In the ROC analysis to evaluate the efficacy of patients' serum FGL-2 levels in predicting NAFLD, the AUC was determined to be 0.759, with p=0.002. It was observed that when FGL-2 levels were at 41 ng/ml, NAFLD patients could be predicted with a sensitivity of 67% and a specificity of 72%.

In the correlation analysis conducted on the patients, it was determined that FGL-2 was correlated with waist circumference (r=0.52, p=0.001), BMI (r=0.44, p=0.003), TG (r=0.37, p=0.016), and HOMA-IR (r=0.37, p=0.016).

DISCUSSION

The significance of FGL-2 in indicating chronic liver damage associated with viral hepatitis has been demonstrated in animal and human studies, particularly in patients with chronic hepatitis C.[17-22] In a study conducted by Foerster et al.,[22] it was observed that patients with chronic hepatitis C who had elevated serum FGL-2 levels exhibited more pronounced necroinflammatory activity and fibrosis. However, this study reported no association between serum FGL-2 levels and alcoholic-related chronic liver disease patients. In our cross-sectional case-control study, we compared the waist circumference, BMI, TG, HOMA-IR, and serum FGL-2 levels of patients diagnosed with NAFLD through biopsy to a control group. We aimed to elucidate the relationship between biopsy, FGL-2 levels, and the aforementioned metabolic parameters. In our study, where we aimed to determine the severity of NAFLD without a biopsy but solely based on FGL-2 measurements, we found that FGL-2 levels in NAFLD patients were significantly higher than in the control group. We also observed that FGL-2 levels correlated with waist circumference, BMI, TG, and HOMA-IR, independent of histological parameters.

In a study similar to ours, which comprehensively addressed the relationship between NASH and FGL-2, Çolak et al.[23] identified a significant difference between the NAFLD (borderline NASH + definite NASH) group and the control group in terms of FGL-2 levels. They determined that steatosis and the simple steatosis group did not correlate with FGL-2. Our study did not find a correlation between the degree of steatosis and FGL-2. (r=0.24, p=0.2) Additionally, when comparing the simple steatosis group with the control group, we did not find a significant difference in FGL-2 levels. (p=0.14) When the Borderline + Definite NASH group was collectively evaluated and compared with the control group, a significant difference was observed between the groups. (p=0.001) These findings suggest that FGL-2 levels may correlate with inflammation, like the previously demonstrated relationship with necroinflammation in chronic HCV rather than fibrosis.

The study conducted by Çolak and colleagues demonstrated that the predictive value of FGL-2 levels in forecasting NAFLD was high. Similarly, in our research,

we found that FGL-2 levels were effective in predicting NAFLD. (AUC=0.759, p=0.002)

Discussing the limitations of our study

Given the cross-sectional nature of our study, a long-term follow-up is necessary to discuss the relationship between histopathological findings and inflammation definitively. Moreover, our inability to show a correlation between transaminases and FGL-2 levels requires explanation. This situation might lead to an optimistic interpretation that FGL-2 levels could reflect the microinflammation level while suggesting that other confounding factors, such as medication, might not have been thoroughly evaluated.

When patients were divided into subgroups, the number of patients appeared marginal for a robust statistical analysis. A larger patient group is necessary for a reliable evaluation. The fact that all patients in our study group are of Turkish origin and belong to the same ethnic group could pose a disadvantage. Lastly, a noticeable deficiency is a lack of staining for FGL-2 in biopsy samples and the unexamined relationship between disease activity in the same preparation.

Conclusion

FGL-2 levels in NASH patients may reflect the balancing role of regulatory T cells and could indicate hepatic damage at the microinflammation level. Estimating disease severity, observing disease progression without biopsy, selecting patients for aggressive treatment approaches, and tightening follow-up intervals for cirrhosis and HCC could be significant. Moreover, we wish to emphasize in our study that FGL-2 could predict metabolic problems and potentially be a target in preventing metabolic liver diseases. For FGL-2 to become a non-invasive prognostic marker that obviates the need for a biopsy, comprehensive and long-term follow-up studies are needed in this field, along with standard measurements in both tissue and serum and transitioning to a cost-effective structure.

Informed Consent

Retrospective study.

Peer-review

Externally peer-reviewed.

Authorship Contributions

Concept: D.I.; Design: D.I.; Supervision: D.I.; Fundings: D.I.; Materials: D.I.; Data: D.I.; Analysis: D.I.; Literature search: D.I.; Writing: D.I.; Critical revision: D.I.

Conflict of Interest

None declared.

REFERENCES

1. World Health Organization. World Health Statistics Report 2011- Cause specific mortalitiy and morbidity. Available at: https://iris.who.int/bitstream/handle/10665/44552/9789241564199_eng.pdf?sequence=1. Accessed May 30, 2024.
2. World Health Organization. The analytic review of integrated management of childhood illness Strategy - Final report. Available at: https://www.who.int/publications/i/item/9241591730. Accessed May 30, 2024.
3. Karron RA, Collins PL. Parainfluenza Viruses In: Fields BN, Knipe DM, Howley PM, editors. Fields virology. 3th ed.Philadelphia: Lipponcott-Raven Publishers; 1996.
4. Younossi ZM, Diehl AM, Ong JP. Nonalcoholic fatty liver diseases:an agenda for clinical research. Hepatology .2002;35(4): 746-752.
5. Brunt EM, Elizabeth M. Nonalcoholic steatohepatit: Definition and pathology. Semınars In Lıver Dısease 2001; 21: 3-16.
6. Brandenburg AH, Groen J, van Steensel-Moll HA, Claas EC, Rothbarth PH, Neijens HJ, et al. Respiratory syncytial virus specific serum antibodies in infants under six months of age: Limited serological response upon infection. J Med Virol 1997;52:97–104.[CrossRef]
7. Eriksson M, Forsgren M, Sjöberg S, Von Sydow M. Respiratory syncytial virüs infection in young hospitalized children. Acta Pediatr Scand 1993;72:47–51.[CrossRef]
8. Moura FE, Nunes IF, Silva GB Jr, Siqueira MM. Respiratory syncytial virus infections in northeastern Brazil: Seasonal trends and general aspects. Am J Trop Med Hyg 2006;74:165–7.[CrossRef]
9. Bryan DL, Hart PH, Forsyth KD, Gibson RA. Immunomodulatory constituents of human milk change in response to infant bronchiolitis. Pediatr Allergy Immunol 2007;18:495–502.[CrossRef]
10. Sami H, Seçil A, Yalçın Ç, Serdar Ö, Esra Ş, Tolga E. Alt solunum yolu enfeksiyonu tanısıyla hastanemize yatırılan olgularda RSV enfeksiyonu sıklığı ve klinik özellikleri. Düzce Tıp Fak Derg 2009;11:38–44.
11. Özacar T, Zeytinoğlu A, Özdoğru E, Aydemir Ş, Tanaç R, Bilgiç A. Alt solunum yolu enfeksiyonu olan çocuklarda respiratuvar sinsityal virüs antijenlerinin araştırılması. İnfeks Derg 1996;10:25–7.
12. Rossi GA, Medici MC, Merolla R; Osservatorio VRS Study Group. Incidence of respiratory syncytial virus positivity in young Italian children referred to the emergency departments for lower respiratory tract infection over two consecutive epidemic seasons. Infection 2005;33:18–24. [CrossRef]
13. Yusuf S, Piedimonte G, Auais A, Demmler G, Krishnan S, Van Caeseele P, et al. The relationship of meteorological conditions to the epidemic activity of respiratory syncytial virus. Epidemiol Infect 2007;135:1077–90. [CrossRef]
14. Levy GA, Leibowitz JL, Edgington TS. Induction of monocyte procoagulant activity by murine hepatitis virus type 3 parallels disease susceptibility in mice. J Exp Med 1981;154:1150–63. [CrossRef]
15. Shalev I, Wong KM, Foerster K, Zhu Y, Chan C, Maknojia A, et al. The novel CD4+CD25+ regulatory T cell effector molecule fibrinogen-like protein 2 contributes to the outcome of murine fulminant viral hepatitis. Hepatology 2009;49:387–97. [CrossRef]
16. Sanyal AJ; American Gastroenterological Association. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology 2002;123:1705–25. [CrossRef]
17. Doolittle RF. The structure and evolution of vertebrate fibrinogen. Ann N Y Acad Sci 1983;408:13–27. [CrossRef]
18. Herman AE, Freeman GJ, Mathis D, Benoist C. CD4+CD25+ T regulatory cells dependent on ICOS promote regulation of effector cells in the prediabetic lesion. J Exp Med 2004;199:1479–89. [CrossRef]
19. Marazzi S, Blum S, Hartmann R, Gundersen D, Schreyer M, Argraves S, et al. Characterization of human fibroleukin, a fibrinogen-like protein secreted by T lymphocytes. J Immunol 1998;161:138–47. [CrossRef]
20. Chan CW, Kay LS, Khadaroo RG, Chan MW, Lakatoo S, Young KJ, et al. Soluble fibrinogen-like protein 2/fibroleukin exhibits immunosuppressive properties: Suppressing T cell proliferation and inhibiting maturation of bone marrow-derived dendritic cells. J Immunol

2003;170:4036–44. [CrossRef]

21. Shalev I, Liu H, Koscik C, Bartczak A, Javadi M, Wong KM, et al. Targeted deletion of fgl2 leads to impaired regulatory T cell activity and development of autoimmune glomerulonephritis. J Immunol 2008;180:249–60. [CrossRef]
22. Foerster K, Helmy A, Zhu Y, Khattar R, Adeyi OA, Wong KM, et al. The novel immunoregulatory molecule FGL2: A potential biomarker for severity of chronic hepatitis C virus infection. J Hepatol 2010;53:608–15. [CrossRef]
23. Colak Y, Senates E, Ozturk O, Yilmaz Y, Coskunpinar E, Kahraman OT, et al. Plasma fibrinogen-like protein 2 levels in patients with non-alcoholic fatty liver disease. Hepatogastroenterology 2011;58:2087–90. [CrossRef]

Nonalkolik Steatohepatitte Fibrinojen-Like Protein-2 (FGL-2) Düzeyinin Klinik Önemi

Amaç: Non-alkolik steatohepatit(NASH) karaciğerin en sık görülen metabolik hastalıklarından biridir. NASH hastalarında tanıdan sonraki on yıllık dönemde yaklaşık %25 oranında siroza gidiş izlenebilmektedir ve kriptojenik sirozun en sık nedeni olarak kabul edilmektedir. Fibrinojen like protein -2 (FGL-2), regülatuar T hücrelerince sekrete edilen fibrinojen süperfamilyasının bir alt üyesidir. Dendritik hücrelerin maturasyonunu engeller ve B hücrelerinin apoptozunu indükleyerek hem doğuştan hem de adaptif immunitenin regulasyonunda görev alır. Yüksek serum FGL-2 düzenin viral hepatitli hastalarda kötü prognostik gösterge olduğu yakın dönem çalışmalarda gösterilmiştir.

Gereç ve Yöntem: Çalışmaya biyopsi ile tanı konulan 30 NASH hastası ve kontrol koluna da 21 sağlıklı vaka dahil edildi. Her iki gruba dahil olan bireylerin serum FGL-2 düzeyleri karşılaştırıldı. Yine her iki gruba dahil edilen bireylerin klinikopatolojik özellikleri ile FGL-2 düzeyleri arasındaki ilişki incelendi.

Bulgular: Serum FGL-2 düzeyleri NASH hastalarında 47±12 ng/ml iken kontrol grubunda 37±10 ng/ml saptandı. İstatistiksel değerlendirme Mann-Whitney U testi ile yapıldı. Biyopsiyle basit yağlanma, border-line NASH, kesin NASH olarak sınıfladığımız hastalar ile kontrol grubu arasında anlamlı fark saptandı (p=0.002). Bu dört grubu kendi arasında değerlendirdiğimizde de aralarında anlamlı fark gördük (Kruskal – Wallis test, p=0.012).

Sonuç: Çalışmamızda NASH tanılı hastalarda biyopsideki steatohepatit ile uyumlu şekilde serum FGL-2 düzeylerini daha yüksek tespit ettik. Yüksek FGL-2 düzeyleri hepatik hasarı mikroinflamasyon düzeyinde iken gösterebilir. Hastalık şiddetinin tahmini, hastalık seyrinin biyopsisiz izlemi ve kontrolü, tedavi açısından agresif yaklaşılacak hastaların seçimi, siroz ve HCC takip aralıklarının sıklaştırılması açısından önemli olabilir. Yine de bu konuda yapılacak daha kapsamlı çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: FGL-2 düzeyi; nonalkolik steatohepatit; prognostik önem.