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Research Article

Non-Invasive Assessment of Liver Fibrosis - ELF test (Enhanced Liver Fibrosis Blood Test) Compared with FibroScan

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Abstract

Liver fibrosis, as detected in progressive chronic liver diseases (CLD), can be defined as an excess deposition of extracellular matrix (ECM) components associated with parallel matrix degradation and remodelling. Liver fibrosis is the most important risk factor for the development of hepatocellular carcinoma (HCC) therefore accurate assessment of the degree of fibrosis is essential. Although liver biopsy is the gold standard, its invasiveness and high cost represent significant limitations, especially in the adult population. For this reason, non-invasive methods, including serum biomarkers and imaging techniques, are increasingly used in clinical practice as an initial screening approach. The aim of this study was to evaluate the diagnostic role of the Enhanced Liver Fibrosis test (ELF-score) in identifying and staging hepatic fibrosis, as well as to analyze its correlation with FibroScan as a non-invasive imaging method. The study included 60 patients with chronic liver disease and 20 participants as the control group, in whom the ELF score was determined by the measuring the following serological biomarkers: hyaluronic acid, amino-terminal propeptide of type III procollagen (PIIINP), and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1). All patients also underwent a FibroScan examination to assess the severity of hepatic fibrosis. Statistical analysis, were performed using SPSS, version 26.0. The results showed a gradual increase in FibroScan values with increasing ELF score, reflecting a positive relationship between these two methods. A strong and statistically significant correlation was identified at the advanced stages of fibrosis, at the early stages the correlation was weaker.

Keywords: ELF Score; FibroScan; Liver Fibrosis; Non-Invasive Liver Fibrosis Test

Introduction

Liver fibrosis is a clinically significant finding that has major impacts on patient morbidity and mortality. The mechanism of fibrosis involves many different cellular pathways, but the major cell type involved appears to be hepatic stellate cells [1]. Liver fibrosis results in accumulation of extracellular matrix (ECM) proteins, mostly collagens Type I and Type III, followed by formation of fibrous scar, which can ultimately compromise normal liver function. Regardless of the etiology, liver fibrosis is characterized by common molecular mechanisms such as hepatocyte death, chronic inflammation with cytokine release, activation of HSCs and disruption of the epithelial or endothelial barrier [2]. Liver fibrosis, which was practically ignored until the 1980s, has become the main topic by identifying stellate cells which are mesenchymal cells located in the space of Disse [3].

Liver fibrosis is the most important risk factor for the development of hepatocellular carcinoma (HCC) and decompensation in patients with chronic liver disease, therefore accurate assessment of the degree of fibrosis is essential [4]. Chronic liver disease is a major public health problem, accounting for 2 million deaths annually and 4% of all deaths worldwide [5]. The prognosis and management of chronic liver disease are highly dependent on the extent and progression of liver fibrosis and the risk of development of cirrhosis and its complications [6]. Patients with chronic liver disease may remain asymptomatic for decades and consequently are often diagnosed at a late stage once complications of cirrhosis have occurred and treatment is less effective [7].

Currently, liver biopsy is still frequently used as the reference standard for fibrosis assessment; however, it is an invasive procedure associated with patient discomfort and, in rare cases, with serious complications [8]. The limitations of liver biopsy have led to the development of non-invasive tests, which have revolutionized the practice of hepatology by allowing the diagnosis of advanced fibrosis or cirrhosis in patients before liver complications develop [9]. Liver fibrosis stage is according to the METAVIR scoring system: F0-F1 as low risk and F2-F4 as high risk [10]. The METAVIR score is used for staging and monitoring liver fibrosis and inflammation, with the following classification: F0 - no fibrosis; F1 - portal fibrosis without septa; F2 - portal fibrosis with few septa; F3 - numerous septa without cirrhosis; and F4 - cirrhosis [11].

The Enhanced Liver Fibrosis test (ELF-score) is based on three serum markers of matrix turnover: tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), hyaluronic acid (HA), and amino terminal peptide of pro-collagen III (PIIINP) [12]. The stage of liver fibrosis is widely recognized as an important predictor of clinical outcomes related to liver disease in patients with chronic liver conditions, regardless of etiology. Studies have shown that the ELF score correlates with histological findings in various chronic liver diseases. These findings suggest that ELF-score can be used as an effective surrogate marker for determining the stage of liver fibrosis [13]. FibroScan (transient elastography) is a non-invasive method used to assess liver fibrosis in patients with chronic liver diseases by measuring liver stiffness. The method is easily performed, providing immediate results and good reproducibility. FibroScan has been validated for the diagnosis of significant fibrosis and cirrhosis [14].

Study Aim

The main aim of this study is to analyze the ELF score as a predictor of outcomes related to liver diseases or progression to fibrosis by analyzing its biochemical components HA, PIIINP, and TIMP-1. The results obtained from the ELF score will be compared with Fibroscan a non-invasive method that measures the elasticity of liver tissue. Fibroscan is a widely evaluated method and has been shown in many studies to be an accurate method for detecting advanced stages of fibrosis and cirrhosis.

Materials and Methods

The study was designed to evaluate non-invasive assessment of liver fibrosis - ELF-score (Enhanced Liver Fibrosis Blood Test) compared with FibroScan and their diagnostic role in the indentification and staging of liver fibrosis. This study represents a diagnostic-agreement study including 80 participants, 60 patients with NAFLD/NASH-associated hepatic fibrosis or cirrhosis and 20 healthy control participants, in whom the ELF score was determined by the measuring the following serological biomarkers: hyaluronic acid, amino-terminal propeptide of type III procollagen (PIIINP), and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1).

All patients also underwent a FibroScan examination to assess the severity of hepatic fibrosis. The study was conducted at the University Institute of Clinical Biochemistry-Skopje, where the

serum non-invasive test ELF Score (Enhanced Liver Fibrosis Score) was performed in collaboration with the University Clinic of Gastroenterohepatology-Skopje, where the non-invasive imaging method FibroScan (transient elastography) was performed. The age of the patients included in the control group ranged from 19 to 57 years. The mean age was 36.00 ± 10.46 years, while the median age was 32.5 years. The age of the patients included in the patient group ranged from 18 to 83 years. The mean age was 55.08 ± 16.62 years, while the median age was 60 years.

For determination of the Enhanced Liver Fibrosis (ELF) score, venous blood samples were collected from participants and processed according to the laboratory's standardized procedures. Serum was separated by centrifugation and its was performed with the Atellica IM analyzer (Siemens) using the direct chemiluminescent method. Laboratory quality-control procedures were applied throughout the analytical process, including internal quality-control procedures and calibration procedures. Samples that did not meet the predefined laboratory quality requirements were repeated. The ELF score test represents a combination of serum biomarkers including HA (hyaluronic acid), PIIINP (amino-terminal propeptide of type III procollagen), and TIMP-1 (tissue inhibitor of matrix metalloproteinases). The ELF score is calculated using the following equation: ELF = 2.278 + 0.851 ln (HA) + 0.751 ln (PIIINP) + 0.394 ln (TIMP-1). Concentrations (C) of each of the constituents are in ng/mL.

Liver stiffness was measured using a FibroScan Mini 430 (Echosens, Paris, France) by vibration-controlled transient elastography, using the M probe. All examinations were performed by a physician specialized and trained in transient elastography. Participants were required to fast for a minimum of 4 hours before the examination. For each participant, 10-15 valid measurements were obtained. The FibroScan examination and blood sampling for ELF score determination were performed on the same day, with an interval of approximately 2 hours between the two procedures.

Participants were recruited from University Clinic for Gastroenterohepatology in Skopje between 2025 and 2026. Eligible participants were adults aged ≥18 years who agreed to participate in the study.

- **Inclusion criteria:** Adult patients (≥18 years) with a confirmed diagnosis of chronic liver disease characterized by hepatic fibrosis associated with non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH)
- **Exclusion criteria**: Patients aged <18 years, pregnant women, patients with acute medical conditions and patients with ascites that precluded reliable FibroScan assessment were excluded from the study.

ELF Score	Interpretation	Clinical Significance
< 9.0	None to mild fibrosis	Low risk of disease progression
≥ 9.0	Moderate fibrosis	Clinically significant fibrosis; requires monitoring and possible treatment
≥ 9.8	Advanced fibrosis to cirrhosis	High risk of complications (portal hypertension, HCC)
≥ 11.3	Cirrhosis	Very high risk; requires surveillance for decompensation and HCC

Table 1: Diagnostic Interpretation of ELF Score [15].

For the processing of the study data, the SPSS software package, version 26.0 for Windows (SPSS, Chicago, IL, USA), was used. For quantitative analysis, measures of central tendency were applied. A two-tailed statistical analysis was performed, and a p-value of < 0.05 was considered statistically significant.

Ethics and confidentiality

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee, Faculty of Medical Sciences, Goce Delcev University, Stip, North Macedonia under approval number 2005-180/8 on 18 June 2026. Written informed consent was obtained from all participants before their inclusion in the study. All participants were informed about the purpose and procedures of the study, and their participation was voluntary.

Results

The study represents a diagnostic-agreement study conducted at the Faculty of Medical Sciences, Goce Delcev University, Stip, North Macedonia, in collaboration with the University Institute

for Clinical Biochemistry at the Mother Teresa University Clinical Center, where the serum non-invasive test ELF Score (Enhanced Liver Fibrosis Score) was performed, and the University Clinic for Gastroenterohepatology in Skopje, also part of the Mother Teresa University Clinical Center, where the non-invasive imaging method FibroScan (transient elastography) was performed. For the statistical analysis, measures of central tendency were used, including the mean, median, minimum values, and maximum values, as well as the measures of variability/dispersion, including standard deviation and standard error. A two-tailed analysis was performed to determine statistical significance.

N	Mean	Median	SD	Min	Max	Percentiles		
						25th	50th	75th
20	36.00	32.5	10.46	19	57	29.75	32.5	45.0
						25th	50th	75th
60	55.08	60	16.62	18	83	43.0	60	67.75

Table 2: Age distribution of control (N = 20) and of patient group (N = 60) included in the study.

The age of the patients included in the control group ranged from 19 to 57 years. The mean age was 36.00 ± 10.46 years, while the median age was 32.5 years. Percentile values indicate that 25% of the patients were younger than 29.8 years, and 75% were younger than 45.0 years, resulting in an interquartile range (IQR) of 15.25 years. The small difference between the mean and the median suggests a relatively symmetric age distribution, without pronounced extreme values, supporting the use of parametric statistics for describing this variable. The age of the patients included in the patient group ranged from 18 to 83 years. The mean age was 55.08 ± 16.62 years, while the median age was 60 years. Percentile values indicate that 25% of the patients were younger than 43.0 years, and 75% were younger than 67.75 years, resulting in an interquartile range (IQR) of 24.75 years. The small difference between the mean and the median suggests a relatively symmetric age distribution, without pronounced extreme values, supporting the use of parametric statistics for describing this variable.

Variable	Control group (n = 20)	Patient group (n = 60)	P-value
Age, mean ± SD (years)	36.0 ± 10.5	55.1 ± 16.6	P < 0.0001
Median age (range)	32.5 (19-57)	60.0 (18-83)	
Female, n (%)	14 (70.0%)	25 (41.7%)	P = 0.039
Male, n (%)	6 (30.0%)	35 (58.3%)	

Table 3: Age and gender distribution of the study groups.

From this table we understand that the patient group was significantly older compared with the control group (55.1 ± 16.6 vs. 36.0 ± 10.5 years, P < 0.0001). Gender distribution also differed significantly between groups, with a higher proportion of males in the patient group (58.3%) compared with controls (30.0%, P = 0.039).

	Control group					Patient group					
	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD	P-value
ELF-score	20	7.72	9.66	8.67	0.62	60	7.52	13.41	9.66	1.14	P = 0.0004
HA	20	9.98	103	34.27	27.25	60	10.06	1000	107.7	174.1	P = 0.0648
PIIINP	20	5.11	13.10	7.78	2.17	60	3.04	39.04	10.59	6.13	P = 0.0488
TIMP1	20	93.30	297.0	202.9	40.54	60	128.0	574.0	255.6	86.40	P = 0.0103
FibroScan	20	2.40	9.10	4.45	1.509	60	3.00	75.0	14.92	13.83	P = 0.0012

Table 4: Comparison of ELF-score, HA, PIIINP, TIMP-1, and FibroScan by control and patient groups.

The table presents a comparison of serological biomarkers of liver fibrosis (ELF-score, HA, PIIINP, TIMP-1) and FibroScan values between control and patient groups. There are significant differences between the control and patient groups in most fibrosis-related markers, indicating higher fibrosis activity in patient group. The mean ELF-score values were higher in patients (8.67 ± 0.62 vs. 9.66 ± 1.14), statistically significant (p = 0.0004), while HA showed much higher mean in patient group (34.27 ± 27.25 vs. 107.7 ± 174.1), not statistically significant (p = 0.0648), large variability (SD = 174.1) weakens significance. Significant differences were observed for PIIINP according to control and patient group (7.78 ± 2.17 vs. 10.59 ± 6.13), statistically significant (p = 0.0488) indicates increased collagen turnover/fibrogenesis, while TIMP1 values showed higher mean in patient group (202.9 ± 40.54 vs. 255.6 ± 86.40), statistically significant (p = 0.0103) who reflects reduced matrix degradation, fibrosis progression. As well as for FibroScan measurements showed much higher values in patient group (4.45 ± 1.50 vs. 14.92 ± 13.83), highly significant (p = 0.0012) strong evidence of increased liver stiffness (fibrosis). According to these results ELF-score, PIIINP, TIMP-1 and FibroScan shows significantly higher fibrosis markers while HA shows a non-significant trend, likely due to high variability. Patient group demonstrated significantly higher ELF scores, PIIINP, TIMP-1 levels, and FibroScan values compared to control group, indicating increased fibrosis activity. Although hyaluronic acid levels were higher in patients, the difference did not reach statistical significance. These findings suggest a greater fibrosis burden in the patient group, although age differences between groups may act as a confounding factor.

Group	Pearson's r	P-value	Significance	Interpretation
Control Group	-0.03	0.909	Not significant	No correlation
Patient Group	0.52	<0.0001	Highly significant	Moderate positive correlation

Table 5: Pearson correlation between ELF score and FibroScan.

According this table Pearson correlation analysis demonstrated no significant correlation between ELF score and FibroScan measurements in the control group (r = -0.03, P = 0.909). In contrast, a statistically highly significant moderate positive correlation was observed in the patient group (r = 0.52, P < 0.0001), indicating that higher ELF scores were associated with higher FibroScan values.

Parameter	Control Median (IQR)	Patient Median (IQR)	U	p-value	Interpretation
ELF-score	8.52 (8.23-9.26)	9.56 (8.98-10.18)	276.5	<0.001	Highly significant
HA ng/mL	21.87 (17.30-45.90)	61.94 (36.80-100.50)	256.0	<0.001	Highly significant
PIIINP ng/mL	7.01 (6.25-9.16)	8.57 (6.80-12.75)	423.5	0.0505	Borderline
TIMP-1 ng/mL	199.50 (176.75-214.00)	237.50 (196.50-294.50)	348.0	0.0052	Significant
FIBROSCAN kPA	4.10 (3.50-4.97)	9.30 (6.57-17.73)	134.0	<0.001	Highly significant

Table 6: Mann-Whitney U test for ELF-score, HA, PIIINP, TIMP-1, and FibroScan of the study groups.

The Mann-Whitney U test analysis showed significant differences between control and patient groups for ELF-score, HA, TIMP-1, and FibroScan, indicating increased fibrosis activity in patient group. FibroScan showed the strongest difference. PIIINP demonstrated borderline significance, suggesting a weaker association. Using the Mann-Whitney U test, significant differences between control and patient groups were observed for ELF score (p = 0.0003), hyaluronic acid (p = 0.0001), TIMP-1 (p = 0.0052), and FibroScan (p < 0.000001), while PIIINP showed borderline significance (p = 0.0505).

Discussion

At present, the serum biomarker ELF score and Fibroscan are the most intensively evaluated non-invasive methods for the assessment of liver fibrosis. The results of the two non-invasive methods using different approaches were comparable for the

diagnosis of significant fibrosis and cirrhosis in the present study. The ELF test was developed in an international multicenter cohort study with 1021 subjects with chronic liver disease using discriminant analysis to identify the above-mentioned algorithm having investigated specific markers of matrix turnover as well as indirect markers of liver function [8]. Most studies that proposed ELF test as a good non-invasive alternative to liver biopsy have focused primarily on populations with HCV infection or nonalcoholic fatty liver disease or those with mixed etiologies including viral hepatitis, primary biliary cirrhosis, etc. [16]. ELF-score is excellent accuracy for advanced fibrosis, but elastography is the strongest diagnostic tool when a reliable measurement can be obtained. Serum markers of fibrosis can rule out advanced fibrosis in primary care [17]. Use of the ELF test to screen for liver fibrosis can reduce the number of futile referrals compared to Fibroscan alone. The sequential combination of Fibroscan followed by ELF in indeterminate cases further minimizes the number of futile referrals, and saves cost, making this a promising future referral strategy [18]. Cossiga., *et al*. 2022, in their study, suggested the use of the ELF test as a screening tool in healthcare. If the ELF results are negative, the risk of advanced fibrosis is low. Conversely, if the ELF test indicates the presence of advanced liver fibrosis, the patient should be referred to the hospital for further invasive procedures [19]. The data from our study shows a positive correlation between ELF score and FibroScan values, where a gradual increase in ELF is associated with increasing severity of liver fibrosis. The patient group was significantly older compared with the control group (55.1 ± 16.6 vs. 36.0 ± 10.5 years). Gender distribution also differed significantly between groups, with a higher proportion of males in the patient group (58.3%) compared with controls (30.0%).

According to this study there are significant differences between the control and patient groups in most fibrosis-related markers, indicating higher fibrosis activity in patient group. These findings support the use of FibroScan as the most reliable non-invasive method, while serum biomarkers may provide complementary information in the assessment of liver fibrosis. FibroScan provides structural assessment of liver stiffness, while serum biomarkers reflect fibrogenesis and extracellular matrix turnover. The combination of these approaches improves overall diagnostic accuracy. These results support the use of ELF score as a reliable indicator of fibrosis stage and the potential for its combination with FibroScan for a more complete non-invasive assessment. In agreement with Durusoy., *et al*. our study demonstrated significantly elevated ELF scores in patients with liver fibrosis compared with healthy controls [20]. Consistent with Friedrich-Rust., *et al*. and Kim., *et al*. ELF score showed a positive correlation with FibroScan measurements, supporting its use as a surrogate marker of liver stiffness [8,16]. This study emphasizes that the implementation of the ELF-score and Fibroscan could potentially be used to monitor treatment efficacy.

Conclusion

ELF-score is a promising technique to identify patients with liver fibrosis, especially for the clarification of significant and/or severe fibrosis. The results of this study indicate that the ELF score represents a reliable non-invasive method for the assessment and staging of liver fibrosis. A statistically significant correlation was observed between ELF score and FibroScan in advanced fibrosis, confirming the compatibility of these two methods. These findings support previous studies validating ELF score as a reliable non-invasive biomarker of hepatic fibrosis and reinforce its potential clinical utility as an alternative or adjunct to elastography in fibrosis assessment. Overall, the ELF score can be used as a valuable tool in clinical practice for non-invasive screening and monitoring of liver fibrosis, reducing the need for liver biopsy.

Conflict of Interest

The authors declare that there are no financial, personal, or institutional conflicts of interest that could have influenced the conduct of this study, the data analysis, or the interpretation of the results. This research was carried out within a strictly academic and scientific framework, without external funding that could introduce bias.

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