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ORIGINAL ARTICLE

Reference Values of Certain Serum Indicators of Liver Fibrosis

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SUMMARY

Background: **Liver fibrosis shows a continuously increasing trend worldwide, due to alcohol abuse, obesity, and, to a lesser extent, chronic hepatitis B and C. Biopsy is still considered the "gold standard" for diagnosis of liver fibrosis. However, it has a number of limitations, such as invasiveness, high cost, need for specialists to conduct and interpret biopsy results, risk of complications, inability to dynamically monitor the pathological process, low patient compliance, and uneven fibrosis distribution. Therefore, there is an increasing demand for non-invasive serum markers that are characterized by easy implementation, low cost, possible repeatability, and high patient compliance.**

Methods: **For a period of two years, 82 clinically healthy, middle aged subjects, mean age 40.5 ± 12.37 years, range 21 - 67 years, were studied. The group was tested for platelet count, prothrombin time, and the following biochemical parameters: Cholesterol - total, HDL, LDL; AST; ALT; GGT; total bilirubin, alfa-2-macroglobulin; haptoglobin and ELF (Enhanced Liver Fibrosis).**

Results: **Reference values of a large number of serum indicators of liver fibrosis are disputable and unspecified. A direct proportional and moderate correlation was found between the BMI and AST, ALT, INR, APRI, GPRI, and Forns Index.**

Conclusions: **We present our original reference values for ELF, AST/ALT, ARPI, GRPI, Fib 4, and Forns Index in 82 clinically healthy subjects.**

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KEY WORDS

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INTRODUCTION

There are three main causes of liver fibrosis (LF) [1, 2,3]:

a) Alcohol abuse and development of alcoholic liver disease and alcoholic steatohepatitis;
b) Non-alcoholic fatty liver disease (NAFLD);
c) Chronic hepatitis B and C.

While the first two etiologic factors are increasing in developed countries, chronic hepatitis is decreasing. In less developed countries the trend is reversed.

The diagnosis of LF is based on the combined use of liver biopsy, imaging techniques, and non-invasive serum biomarkers. The use of liver biopsy has a number of limitations, such as invasiveness, high cost, need for specialists to conduct and interpret biopsy results, risk of complications, inability to dynamically monitor the pathological process, and low patient compliance [3,4].

Imaging techniques are very expensive and with limited use

Non-invasive serum markers are becoming more widely used because they are feasible, have low cost, can be monitored dynamically, and are readily accepted by the patients. In the literature, there is little data on reference values for these indicators in healthy subjects. For this reason, we conducted this study with the aim to construct reference values of direct and indirect markers for LF (ELF, AST/ALT, APRI, GPRI, Fib 4, and Forns Index) in 82 clinically healthy subjects.

MATERIALS AND METHODS

For a period of two years, 82 clinically healthy, middle-aged subjects, mean age 40.5 ± 12.37 years, range 21 - 67 years, were studied. Of these, 41 (50%) were men with a mean age of 38.37 ± 11.15 years, range 21 - 61 years, and 41 (50%) were women with a mean age of 42.80 ± 13.24 years, range 23 - 67 years (Figure 1). The subjects voluntarily consented to participate in the study. They had no complaints, received no medications and supplements, and presented no abnormalities in the routine tests of urine and blood count. After an overnight fast, the subjects, included in the group, were tested for platelet count (on the analyzer CD Ruby System, Abbott Diagnostics, original reagents and control materials), prothrombin time (on the Sysmex CA1500 System), and the following biochemical parameters (on the analyzer Architect c8000 System, Abbott Diagnostics, original reagents, controls and calibrators): apo A1, glucose, total cholesterol, HDL cholesterol, LDL cholesterol, total bilirubin (TBILI), albumin (ALB), alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alfa 2 macroglobulin. The enzymatic activity was determined at 37°C, according to the International Federation of Clinical Chemistry Standards.

The following formulas were used to determine the indicators (1 - 4):

AST [U/L]/ALT [U/L] – ratio,

APRI = [(AST [U/L]/upper limit of normal)/platelet count 10^9/L] x 100,

GPRI =[(GGT[U/L]/upper limit of normal/platelet count 10^9/L] x 100,

Forns Index = [7.811 - 3.131 x ln (platelet count 10^9/L)] + [0.781 x ln GGT [U/L] + [3.467 x ln (age) - 0.014 x cholesterol [mg/dl]],

(ln: natural logarithm),

Fib 4 = [age (years) x AST [U/L]]/[platelet count 10^9/L x √ALT [U/L]],

ELF_{result} = 2.278 + 0.851 ln (C_{HA}) + 0.751 ln (C_{P3NP}) + 0.394 ln (C_{TIMP1}).

The obtained data were entered and processed with IBM SPSS Statistics 23.0 statistical package. The accepted significance level, at which the null hypothesis was rejected, was $p < 0.05$. Descriptive, variation, graphic, and correlation analyses were used, as well as the non-parametric Kolmogorov-Smirnov and Shapiro-Wilk tests to verify the distribution of normality, Student's *t*-test and the non-parametric Mann-Whitney test to check the hypothesis of differences between two independent samples. The reference ranges were calculated with the RefVal program.

RESULTS

The results obtained are presented in the tables and figures below. Data from the variation analysis of studied indicators are given in Table 1. The distribution of normality is illustrated in Figure 2. Only three of the studied parameters presented with the Gaussian distribution: platelet count, total bilirubin, and Forns Index (Figure 2). The comparative analysis in men and women (Table 2) showed a significant difference ($p < 0.05$) for some of the markers. Men had significantly higher mean values of ELF, APRI, and GPRI, compared to women. There was no significant gender difference for the markers AST/ALT, Fib 4 and Forns Index. The correlation analysis (Table 3) revealed a strong correlation between the age and Forns Index ($r = 0.781$; $p < 0.001$). A direct proportional and moderate correlation was found between BMI and AST, ALT, INR, APRI, GPRI, and Forns Index.

Table 4 shows the resulting reference values for the studied biomarkers under the percentile analysis, conducted with the RefVal program.

DISCUSSION

Non-invasive serum biomarkers are divided into direct (Class I) and indirect (class II) markers.

Direct markers reflect the major part of the pathogenesis of LF, namely, fibrogenesis and fibrolysis. In healthy people, fibrogenesis and fibrolysis of matrix tissue are in balance. Intensified fibrogenesis develops in virtually all patients with chronic liver injury. Direct serum biomarkers reflect the products, derived from the turnover of the extracellular matrix during fibrogenesis and fibrolysis. There is an increase in the serum levels of hyaluronic acid, procollagen IV, C-peptide, tissue inhibitors of metalloproteinases, matrix metalloproteinases, and the like [1,5,12].

Direct markers may be subdivided into enzymatic, collagen, glycoprotein, glycosaminoglycan and matrix-metalloproteinase markers.

Table 1. Variation analysis of the studied quantitative indicators.

Parameter	$\overline{X}$	SD	Median	Min	Max
Age (years)	40.59	12.37	41.00	21.00	67.00
BMI (kg/m2)	24.60	4.10	25.65	15.94	31.60
PLT (109/L)	276.56	60.05	285.00	150.00	420.00
Apo A1 (mg/dL)	204.09	29.68	205.00	121.00	263.00
Glucose	5.32	1.24	5.15	3.50	9.29
Total cholesterol (mg/dL)	208.03	29.85	201.35	155.21	279.92
HDL cholesterol (mg/dL)	51.44	14.85	46.91	35.14	103.86
LDL cholesterol (mg/dL)	109.86	28.69	100.97	42.47	194.98
TBILI (µmol/L)	14.23	4.72	14.00	6.00	25.90
Albumin (g/L)	45.06	4.08	45.00	33.00	55.00
ALP (U/L)	91.44	18.67	98.00	33.00	121.00
AST (U/L)	28.32	9.68	26.00	9.00	56.00
ALT (U/L)	25.95	11.17	22.00	10.00	46.00
GGT (U/L)	29.29	13.18	29.50	9.00	60.00
PT (U/L)	100.11	7.44	99.00	74.00	123.00
INR	1.03	0.06	1.04	0.86	1.39
Alfa-2-macroglobulin (g/L)	263.09	80.15	266.00	99.00	500.00
Haptoglobin (g/L)	1.52	0.80	1.31	0.31	3.22
ELF	6.68	0.58	6.79	5.21	8.12
HA (ng/mL)	4.98	1.01	5.13	3.01	17.04
PIIINP (ng/mL)	5.98	0.66	5.20	2.29	7.06
TIMP 1 (ng/mL)	119.5	36	110	88	152.2
APRI	0.28	0.15	0.25	0.07	0.90
AST/ALT ratio	1.16	0.38	1.05	0.53	3.00
FIB 4	0.84	0.42	0.73	0.30	2.35
GPRI	0.26	0.14	0.23	0.08	0.75
Forns Index	2.59	1.41	2.43	-0.06	5.35

Table 2. Quantitative indicators in men and women.

Parameter	Male (n = 41)		Female (n = 41)		p
	$\overline{X}$	SD	$\overline{X}$	SD	
Age (years)	38.37	11.15	42.80	13.24	0.104
BMI (kg/m^2)	27.25	2.53	21.94	3.63	< 0.001
PLT (109/L)	278.78	68.72	274.28	50.43	0.524
Apo A1 (mg/dL)	198.17	29.18	210.00	29.34	0.127
Glucose (mmol/L)	5.80	1.20	4.83	1.08	< 0.001
Total cholesterol (mg/dL)	213	28	202	31	0.054
HDL cholesterol (mg/dL)	44	6	59	17	< 0.001
LDL cholesterol (mg/dL)	106	23	114	34	0.738
TBILI (µmol/L)	15.99	3.81	12.47	4.92	< 0.001
Albumine (g/L)	46.95	3.54	43.17	3.72	< 0.001
ALP (U/L)	99.00	11.67	83.88	21.27	0.001
AST (U/L)	33.51	9.81	23.12	6.18	< 0.001
ALT (U/L)	31.15	11.51	20.76	8.06	< 0.001
GGT (U/L)	38.37	11.46	20.22	7.17	< 0.001
INR	1.06	0.07	1.01	0.04	< 0.001
Alfa 2 macroglobulin (g/L)	263.78	81.43	262.39	79.86	0.693
ELF	6.84	0.59	6.53	0.54	0.014
APRI	0.33	0.18	0.22	0.08	0.001
AST/ALT ratio	1.10	0.26	1.21	0.46	0.450
FIB 4	0.80	0.34	0.88	0.49	0.948
GPRI	0.31	0.16	0.20	0.09	0.001
Forns Index	2.58	1.50	2.59	1.33	0.984

Table 3. Correlation analysis between age and BMI and some indicators.

Parameter	Age (years)	BMI (kg/m^2)
AST (U/L)	-0.156	0.414 ***
ALT (U/L)	-0.041	0.335 **
GGT (U/L)	-0.110	0.615 ***
INR	0.062	0.415 ***
ELF	-0.135	0.161
APRI	-0.162	0.374 **
AST/ALT ratio	-0.068	-0.164
FIB 4	0.036	0.120
GPRI	-0.033	0.478 ***
Forns Index	0.781 ***	0.310 **

* - p < 0.05, ** - p < 0.01, *** - p < 0.001.

Table 4. Reference values of the main markers.

Parameter	Gender	Percentile	Limit	95% CI	
				Low range	High range
ELF	Male	0.025	5.581	5.182	5.954
		0.975	7.874	7.639	8.094
	Female	0.025	5.452	5.184	5.723
		0.975	7.427	7.253	7.581
APRI		0.025	0.107	0.089	0.127
		0.975	0.625	0.493	0.949
AST/ALT ratio		0.025	0.657	0.602	0.717
		0.975	2.065	1.788	2.477
FIB 4		0.025	0.373	0.340	0.411
		0.975	2.172	*	*
GPRI		0.025	0.096	0.084	0.110
		0.975	0.612	0.491	0.889
Forns Index		0.025	0.253	0.004	0.531
		0.975	5.205	4.826	5.560

CI - confidence interval.
* Calculations of the upper limit are made by applying a non-parametric method and therefore the confidence interval is not presented.

Hepatic fibrogenesis is a dynamic interaction between cellular and molecular processes (Figure 3). Based on the etiology of LF, there is some difference in the pathogenesis of the disease. However, there are many common contact points [2,4,6,7]. The development of LF is a complex process, in which necrosis, apoptosis, inflammation, fibrogenesis, and fibrolysis play a crucial role. There is an increased production of extracellular matrix (ECM), which may be increased 5- to 6-fold [8, 9]. Normally, the ECM is composed of macromolecules, such as collagen I, III, IV, V, and VI, glycoproteins, laminin, fibronectin, proteoglycans, and the like. During intensified fibrogenesis, the matrix is increased at the expense of collagen I and III and additional cellular components. These cells change their structure and function. Some undergo necrosis and apoptosis, others - proliferation and "activation", still others increase the synthesis and secretion of various components, and some are subjected to transformation resulting in myofibroblasts [10,11]. Myofibroblasts originate mainly from

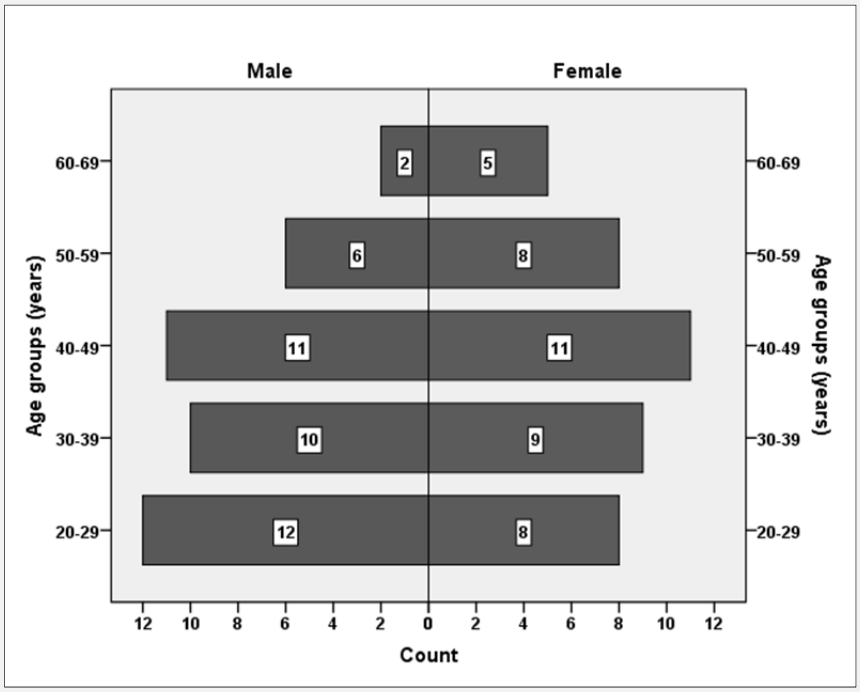

Figure 1. Distribution of the control group by gender and age.

hepatic stellate cells (HSCs). The activation of stellate cells and their transformation into proliferative, fibrogenic, and contractile myofibroblasts is a key event in fibrogenesis. Myofibroblasts synthesize and secrete collagen I, II, and III; adhesive glycoproteins, such as laminin, fibronectin, entactin, vitronectin, tenascin, osteonectin, elastin; proteoglycans - heparin, dermatan, chondroitin sulfates; glycosaminoglycans, such as hyaluronic acid; matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and others [9,10, 12]. In parallel, the body seeks to reduce fibrogenesis through destruction of matrix components. The balance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) is crucial for the ECM homeostasis. Matrix metalloproteinases (MMRs 1 - 13) are an important factor for fibrolysis. They are central in the process of fibrotic tissue remodeling. They cleave fibrillar collagens and make gelatin susceptible to degradation. Liver fibrosis is a consequence of the interaction of a complex network of cytokines, such as TGF-β, IL-1, IL-6, IL-10, IL-17, TNF-α, IFN-α, IFN-β, IFN-γ, PDGF, endothelin-1, reactive oxygen species, and others [8,10,12]. If the harmful agent continues to function, fibrogenesis increases while remodeling decreases. Under the influence of hepatitis viruses and alcohol, hepatocytes and Kupffer cells also stimulate myofibroblasts and fibrogenesis. The following three phases can be distinguished in the development of LF: pre-inflammatory phase with activation of the HSC by the perishing hepatocytes; inflammatory phase, in which the HSCs are further encouraged to trans-differentiate to myofibroblasts, and post-inflammatory phase, in which myofibroblasts secrete stimulating cytokines and ECM components [9,12].

Indirect serum biomarkers are mainly indicators of synthetic function. They are frequently correlated with the signs and symptoms of the disease. They are well-established and routinely used in clinical practice. They can be categorized into enzymes, such as ALT, AST, ALP and GGT, markers of synthetic function, such as PT/INR, bilirubin, haptoglobin, albumin, apolipoprotein A1, α-2-macroglobulin, transferrin and ceruloplasmin, and indirect markers, such as platelets, α1-antitrypsin and ferritin [2-4]. Indirect serum biomarkers are not surrogate markers of fibrogenic processes in the liver. They are usually combined into panels.

Based on the above facts, one direct marker (Enhanced Liver Fibrosis, ELF) and indirect markers (AST/ALT, APRI, GPRI and Forns Index) were selected for the purposes of study. Their choice was determined by their informativeness (diagnostic sensitivity and specificity), accessibility, affordability, and the possibility for wide application [13-15]. The enzymes AST, ALT and GGT

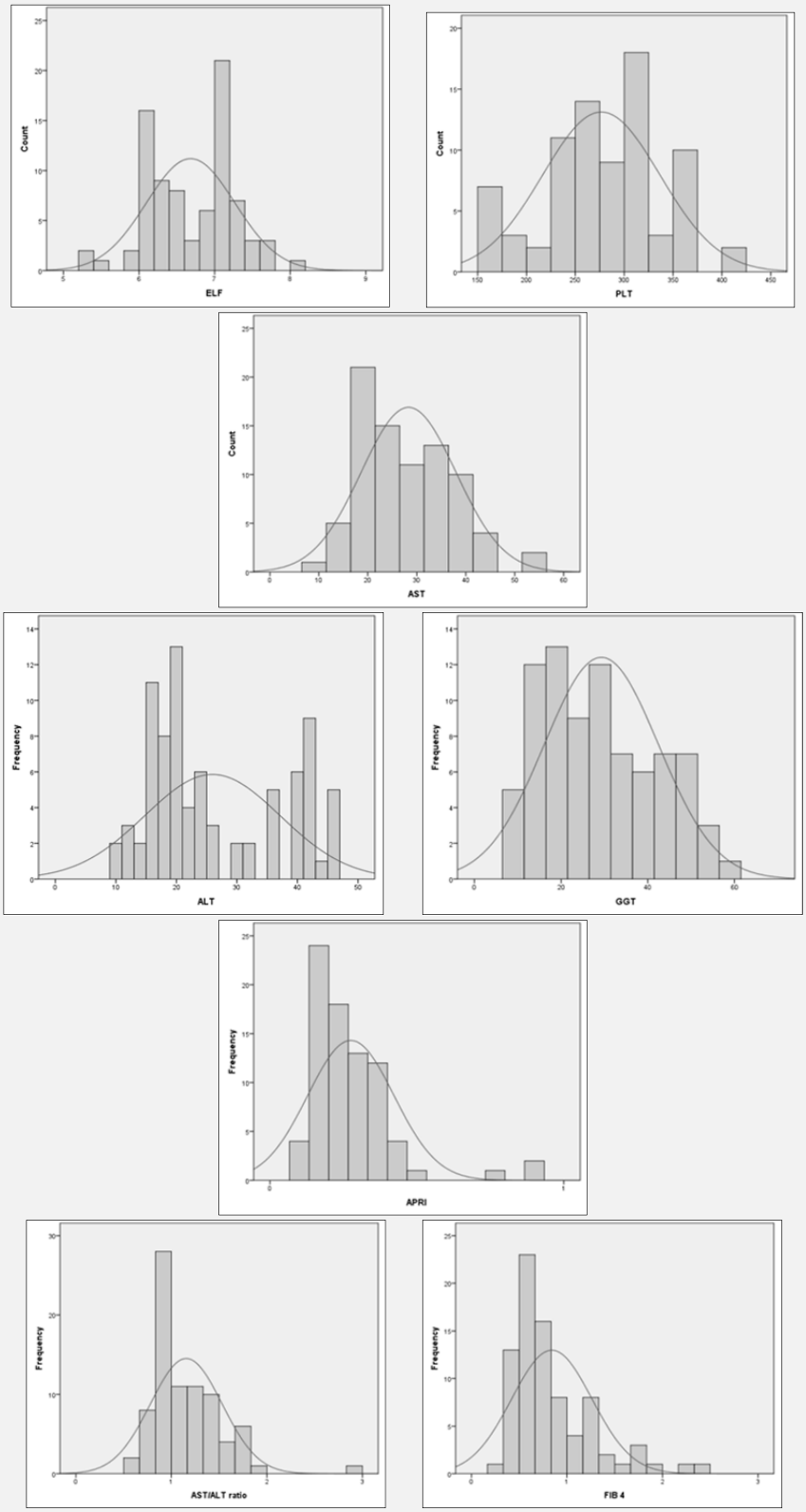

Figure 2. Frequency distribution of the studied population by: ELF, p < 0.001 (a); platelets (b); AST, p = 0.016 (c); ALT, p < 0.001 (d); GGT, p = 0.001 (e); APRI, p < 0.001 (f); AST/ALT ratio p < 0.001 (g); FIB 4, p < 0.001 (h); GPRI, p = 0.001 (i); by Forns Index, p = 0.200 (j).

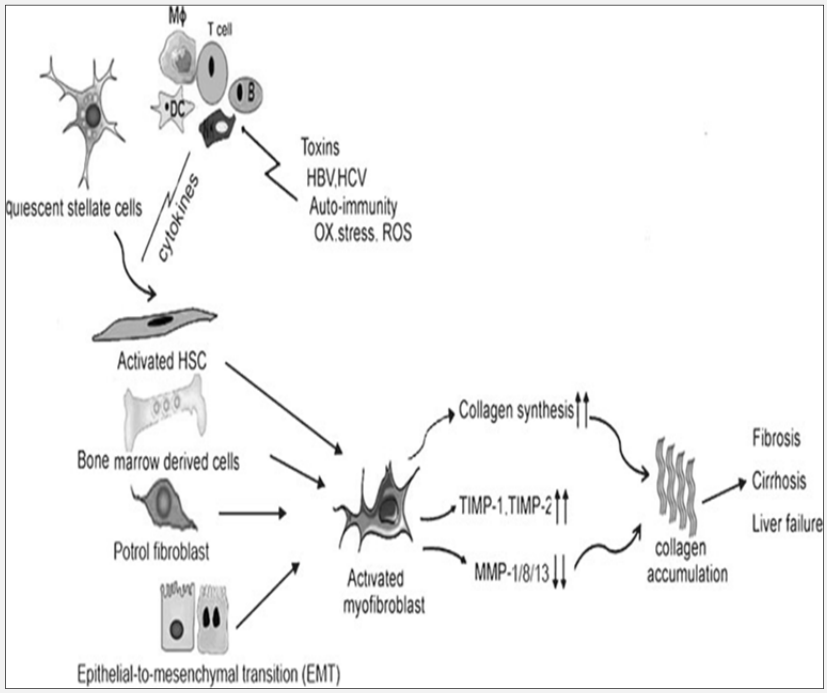

Figure 3. Schematic representation of fibrogenesis (by Xu, 2010, 10).

are well-known and widely used serum markers of liver dysfunction in alcoholism, viral hepatitis C and B, and NAFLD. In the suggested panels, these enzymes demonstrated even more informativeness for LF. The ELF test is an algorithm of three fibrotic markers - HA (hyaluronic acid), PIIINP (N-terminal propeptide of type III collagen), and TIMP-1 (tissue inhibitor of matrix metalloproteinase 1). It was suggested for the first time by the European Association for the Study of the Liver (EASL) in 1997, but has only recently been a subject of investigations [4,15-17]. The test is the first routine, clinically validated direct biomarker.

Our results in clinically healthy persons are in accordance with the scarce literature data [4,16].

Most of the authors investigate non-invasive serum markers in patients in parallel with liver biopsy. They use cutoff values for the diagnosis of various stages of LF [4,18-22]. It is quite understandable that in determining the reference ranges of ELF, AST/ALT, APRI, GPRI, FIB 4, and Forns Index in healthy individuals, we cannot use the same approach. Depending on the type of distribution of the indicators, we applied variation and parametric analyses (Table 1 and 4). We found a Gaussian distribution for the Forns Index only (Table 1). Men had significantly higher mean values of ELF, GGT, INR, APRI and GPRI, and for women the higher values were for HDL-cholesterol and PT (Table 2). Both genders did not differ statistically significantly in terms of the indicators age, platelet count, total cholesterol, LDL, AST/ALT ratio, FIB 4, and Forns Index. From Table 3, it is clear that age correlates strongly and positively only with the Forns Index, and the BMI strongly and positively with AST, GGT, INR, and GPRI. The reference ranges, calculated with the RefVal program by applying a non-parametric method, are presented in Table 4. Our reference values, obtained by applying variation and percentile analyses, are very close. The upper limits of our indicators are quite close to the cutoff values for the same parameters, suggested by most of the authors [4,6,7,18,23-25]. We found only two authors, who reported reference values. According to Lichtinghagen et al. [4], the reference range of ELF is from 6.7 to 9.8 and it is substantially higher for men, compared to women (7.0 - 9.9 vs. 6.6 - 9.3). Zang et al. [16] reported that the reference intervals for APRI, calculated by using a non-parametric analysis, were 0.1398 - 0.6266 for men and 0.1282 - 0.5798 for women. We are convinced that the reference ranges, determined by us in clinically healthy subjects, would help to diagnose LF even in its earliest stages.

CONCLUSION

This study was dedicated to establishing the reference ranges of ELF, AST/ALT, APRI, GPRI, FIB 4, and Forns Index markers in 82 clinically healthy subjects by using variation and percentile analyses.

Declaration of Interest:
The authors have nothing to declare.

References:

1. Cox-North PP, Shuhart MC. Evaluation and Staging of Liver Fibrosis. Last Updated: October 21st, 2015 https://www.hepatitisc.uw.edu/go/evaluation-staging-monitoring/evaluation-staging/core-concept/all.

2. Baranova A, Lal P, Birerdinc A, Younossi ZM. Non-Invasive markers for hepatic fibrosis. BMC Gastroenterology. 2011,11:91 https://bmcgastroenterol.biomedcentral.com/articles/10.1186/1471-230X-11-91.

3. Wang R, ZhangO, Zhao S, et al. Gamma-glutamyl transpeptidase to platelet ratio index is a good noninvasive biomarker for predicting liver fibrosis in Chinese chronic hepatitis B patients. J Int Med Res 2016;44(6):1302-13 http://journals.sagepub.com/doi/pdf/10.1177/0300060516664638.

4. Lichtinghagen R, Pietsch D, Bantel H, Manns MP, Brand K, Bahr MJ. The Enhanced Liver Fibrosis (ELF) score: normal values, influence factors and proposed cutoff values. J Hepatol 2013; 59: 236-42 (PMID: 23523583).

5. Ebrahimi H, Naderian M, Sohrabpour AA. New Concepts on Pathogenesis and Diagnosis of Liver Fibrosis; A Review Article. Middle East J Dig Dis 2016;8:166–78 (PMCID: PMC5045669) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5045669/.

6. Papastergiou V, Tsochatzis E, Burroughs AK. Non-invasive assessment of liver fibrosis. Ann Gastroenterol 2012:25:218-31 (PMCID: PMC 3959378) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3959378/.

7. Fallatah HI. Review Article. Noninvasive Biomarkers of Liver Fibrosis: An Overview. Adv Hepatology 2014;2014:357287 http://dx.doi.org/10.1155/2014/357287.

8. Elpek GO. Cellular and molecular mechanisms in the pathogenesis of liver fibrosis: An update. World J Gastroenterol 2014;20: 7260-6 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4064072.

9. Hernandez-Gea V, Friedman SL. Pathogenesis of Liver Fibrosis: mechanisms of disease. Annu Rev Pathol 2011;6:425-56 (PMID: 21073339).

10. Xu R, Zhang Z, Wang F. Liver Fibrosis: mechanisms if immune mediated Liver Injury. Cell Mol Immunol. 2012;9:296-301 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4132585/.

11. Almpanis Y, Demonakou M, Tiniakos D. Evaluation of liver fibrosis: "Something old, something new" Ann Gastroenterol 2016;29:445-53 https://www.ncbi.nlm.nih.gov/pmc/articles/ PMC 5049550/.

12. Iredale JP, Thompson A, Henderson NC. Extracellular matrix degradation in liver fibrosis. Biochim Biophys Acta 2013;1832: 876-83 (PMID: 23149387).

13. Petersen JR, Stevenson HL, Krishna SK, et al. Evaluation of the APRI (AST, platelet ratio index) and ELF™ (Enhanced Liver Fibrosis) tests to detect significant fibrosis due to chronic hepatitis C. J Clin Gastroenterol 2014;48:370-6 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3947711/.

14. Armstrong MJ, Houlihan DD, Bentham L, et al. Presence and severity of non-alcoholic fatty liver disease in a large prospective primary care cohort. J Hepatol 2012;56:234-40 (PMID: 21703178).

15. Chin JL, Pavlides M, Moolla A, Ryan JD. Non-invasive Markers of Liver Fibrosis: Adjuncts or Alternatives to Liver Biopsy? Front Pharmacol 2016;7:159-68 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4913110/.

16. Zhang GM, Guo XX, Zhu BL, et al. Establishing Reference Intervals of Aspartate Aminotransferase-to-Platelet Ratio Index for Apparently Healthy Elderly. Clin Lab 2016; 62:135-40 (PMID: 27012043).

17. Lin ZH, Xin YN, Dong QJ, et al. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. Hepatology 2011;53:726-36 (PMID: PMC 21319189).

18. Li Y, Chen Y , Zhao Y. The Diagnostic Value of the FIB-4 Index for Staging Hepatitis B-Related Fibrosis: A Meta-Analysis. PLoS One. 2014 Aug 28;9(8):e105728 (PMID: 25165830).

19. Shah AG, Lydecker A, Murray K, et al. Use of the FIB4 index for Non-Invasive Evolution of fibrosis in nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2009;7:1104-12 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3079239/.

20. Fernandes FF, Ferraz ML, Andrade LE, et al. Enhanced liver fibrosis panel as a predictor of liver fibrosis in chronic hepatitis C patients. J Clin Gastroenterol 2015;49:235-41 (PMID: 24714186).

21. Jain P, Tripathi BK, Gupta B, Bhandari B, Jalan D. Evaluation of Aspartate Aminotransferase-to-Platelet Ratio Index as a Non-Invasive Marker for Liver Cirrhosis. J Clin Diagn Res 2015;9: OC22-OC24 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC 4668451/.

22. Stasi C, Milani S. Non-invasive assessment of liver fibrosis: Between prediction/prevention of outcomes and cost-effectiveness. World J Gastroenterol 2016;22:1711-20 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4722001/.

23. Xie Q, Zhou X, Huang P, Wei J, Wang W, Zheng S. The Performance of Enhanced Liver Fibrosis (ELF) Test for the Staging of Liver Fibrosis: A Meta-Analysis. PLoS One 2014;9:2014 (PMID: 24736610).

24. Friedrich-Rust M, Rosenberg W, Parkes J, Herrmann E, Zeuzem S, Sarrazin C. Comparison of ELF, FibroTest and FibroScan for the non-invasive assessment of liver fibrosis. BMC Gastroenterology 2010 Sep 9;10:103 (PMID: 20828377).

25. Khattab M, Sakr MA, FattahMA. Novel non-invasive biological predictive index for liver fibrosis in hepatitis C virus genotype 4 patients World J Hepatol 2016;8:1392-401 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5114475/.