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Original Article

Possibility of non-invasive diagnostics of liver fibrosis in patients after chemotherapy with normal weight and overweight

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Abstract

According to the World Health Organization, diseases of the gastrointestinal tract rank third place after cardiovascular disease and cancer and more than 2 billion people suffer from hepatic diseases and toxic hepatitis is one of them. Given the complexity of differentiation and confirmation of iatrogenic toxic hepatitis and broad spectrum of possible medication side effects, there is a real problem with precise statistics of hepatic drug lesions. This study aims to improve the possibility of diagnosing liver fibrosis in patients with normal weight and overweight and determine and study the relationship between the level of polyfunctional cytokine TGF-β1 with BMI. We examined 44 patients with a history of breast cancer of I-II degree according to the TNM classification (TNM-8 system, 2016) without malignancy and who belonged to the III clinical group (persons with proven malignant tumors who have completed radical treatment and are in remission) aged 35 to 79 years. Patients were divided into two main groups. The first group included patients without signs of toxic liver disease and the second group represented patients with signs of toxic hepatitis. In addition to biochemical analysis, the subjects were examined to determine the FibroTest index to verify the fibrosis stage. Additionally, the FIB-4 index was calculated. This study found that FIB-4 and fibrotest may be sensitive and reliable methods for liver fibrosis screening in patients after cancer chemotherapy. The presence of excess body weight may be an important factor in predicting the development of severe fibrosis in such patients. The level of profibrogenic cytokine TGF-β1 may be an additional predictor of fibrosis progression.

Keywords: toxic hepatitis, fibrosis, breast cancer, overweight, TGF-β1.

Introduction

Gastrointestinal tract diseases are among the most common pathologies in the world population. According to the World Health Organization (WHO), they rank third place after cardiovascular disease and cancer [1, 2], and more than 2 billion people suffer from hepatic diseases.

Chronic diffuse liver diseases include not only chronic hepatitis and cirrhosis of viral etiology but also a large number of nosologies of non-viral origin [3, 4]. We had significant domination of chronic viral origin two three decades ago in Ukraine [5], but now the main reason are metabolic and toxic ones [6]. Non-alcoholic steatohepatitis leads now, but taking into account the prevalence of comorbidity in patients with gastroenterology disorders, toxic liver disease is also very important.

Toxic hepatitis is a diffuse inflammatory process in the liver which is caused by the impact of industrial poisons of hepatotoxic action in doses exceeding the maximum allowable concentration. It is characterized by steatosis, lymphocytic infiltration of the liver lobes and diffuse fibrosis without significant liver architecture changes [7].

Given the complexity of differentiation and confirmation of iatrogenic toxic hepatitis and broad spectrum of possible medication side effects, we have a real

problem with precise statistics of hepatic drug lesions. Data from clinical studies show that in the structure of acute and chronic liver disease, the drug lesions range from 0.7% to 20% [8] and the percentage of all adverse reactions associated with the use of drugs ranges from 2% to 28%, 10% of them are cytostatic [9]. It was found that 20% of patients with toxic liver disease due to drugs with jaundice are at risk of fulminant hepatitis onset [6, 10]. Polychemotherapy with cytostatics and biologics causes profound immunosuppression due to both the tumor and treatment, as well as possible remote profibrogenic effects.

Transforming growth factor β1 (TGF-β1) is a polyfunctional cytokine. It is known that it is currently positioned as the main profibrogenic cytokine, which is actively involved in the formation of liver fibrosis. In addition to initiating the transformation of stellate cells into myofibroblasts [11], it potentiates the expression of matrix genes by inhibiting the production and activity of matrix metalloproteinases and increases the activity of tissue inhibitors and induces apoptosis of hepatocytes by inhibiting their proliferation [12, 13, 14]. All these factors can be assessed as the main signs of hepatic fibrogenesis.

The aim of this study is to improve the possibility of diagnosing liver fibrosis due to its toxic lesion after chemotherapy in patients with normal weight and overweight by analyzing serum markers of fibrosis. Determine and study the relationship between the level of polyfunctional cytokine TGF-β1 with body mass index.

Material and methods

The study was conducted based on the gastroenterology department of Ternopil University Hospital. The diagnosis of toxic liver damage was verified in accordance with the existing EASL and national guidelines. The degree of hepatotoxicity was determined according to the criteria of general toxicity of the US National Cancer Institute (NCCN CTC v 5.0) (Table 1).

According to the classification of the WHO International Group on Obesity (1997), all patients were divided into subgroups: 18.5–24.9 kg/m^2 – normal body weight; 25–29.9 kg/m^2 – overweight (pre-obesity); 30.0–34.9 kg/m^2 – obesity of the I degree. Thus, we examined 44 patients with a history of breast cancer of I-II degree, according to the TNM classification (TNM-8 system, 2016) without malignancy and belonged to the III clinical group (persons with proven malignant tumors who have completed radical treatment and are in remission) aged 35 to 79 years. Patients were divided into two main groups. The first group included 16 (36.4%) patients without signs of toxic liver disease, the second group included 28 (63.6%) patients with signs of toxic hepatitis.

In addition to biochemical analysis, the subjects were examined for serum markers alpha2-macroglobulin, haptoglobin and apolipoprotein A1 to determine the FibroTest index to verify the fibrosis stage. Additionally, the FIB-4 index was calculated.

To analyze the results of FibroTest and transfer the data to the stage of fibrosis, the most common scale of histological indices METAVIR was used (Table 2):

Value FIB-4<1.45 indicated the absence of significant fibrosis (F0-F2 fibrosis), and value FIB-4>3.25 indicated the presence of significant fibrosis (F3-F4 fibrosis).

Another predictor of liver fibrosis progression is serum transforming growth factor – β1 (TGF-β1). Enzyme-Linked Immunosorbent Assay (ELISA) was used to quantify serum TGF-β1 levels in the Multiskan

Table 1: Assessment of hepatotoxicity cccording to the recommendations of the US National Cancer Institute - NCCN CTC v5.0.

Indicator	Degree				
	0	I	II	III	IV
AP, times	Norm	>2.5×UMN	>2.5–5.0×UMN	>5.0–20.0×UMN	>20.0×UMN
Bilirubin, times	Norm	1.0–1.5×UMN	>1.5–3.0×UMN	>3.0–10.0×UMN	>10.0×UMN
GGTP, times	Norm	>2.5×UMN	>2.5–5.0×UMN	>5.0–20.0×UMN	>20.0×UMN
AST, times	Norm	>2.5×UMN	>2.5–5.0×UMN	>5.0–20.0×UMN	>20.0×UMN
ALT, times	Norm	>2.5×UMN	>2.5–5.0×UMN	>5.0–20.0×UMN	>20.0×UMN
Hypoalbuminemia, g/dl	Norm	<3.0 NMN	≥2.0–<3.0	<2.0	-

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Table 1: Continued.

Indicator	Degree				
	0	I	II	III	IV
Hepatic dysfunction/ insufficiency (clinical)	Norm	-	-	Tremor	Encephalopathy or coma
Condition of the portal vein	Norm	-	Decreased blood circulation in the portal vein	Restoration/ retrograde flow in the portal vein	-

FC-357 automated enzyme-linked immunosorbent analyzer according to the instructions of test-system "Human TGF β1 Platinum ELISA (BMS249/4 BMS249/4TEN; eBioscience, Austria)".

Results

All subjects were female (100%). In the first group, the average age was 48.71±2.37 and in the second group, 67.27±1.17 respectively. Both groups were representative in terms of age and gender. When using the FIB-4 index in patients with different BMI, a significant difference was found in the value of the index in patients with normal body weight and overweight ($p<0.01$), normal body weight and grade I obesity ($p<0.01$), excessive body weight and grade I obesity ($p<0.01$). Spearman's correlation analysis shows a significant progression of liver fibrosis, calculated by the FIB-4 index, with increasing BMI ($r=0.79$, $p<0.05$) (Figure 1).

Therefore, analyzing the results of this index, we can assume that person with normal body weight did not show moderate fibrosis, as the calculated average values were less than 1.45. In turn, patients with overweight and I-degree obesity represented the average values that were numerically less than 3.25, which indicates the absence of significant fibrosis.

Table 2: The results of FibroTest using the scale of histological indices METAVIR.

FibroTest	METAVIR Stage of Fibrosis
0.75–1.00	F4
0.73–0.74	F3-F4
0.59–0.72	F3
0.49–0.58	F2
0.32–0.48	F1-F2
0.28–0.31	F1
0.22–0.27	F0-F1

The dependence of FIB-4 on the level of AST ($r=0.69$, $p<0.05$), ALT ($r=0.71$, $p<0.05$), dyslipidemia ($r=0.33$, $p<0.05$), as well as platelets ($r=-0.6$, $p<0.05$). (Table 3).

The Fibrotest method was used to diagnose the stages of liver fibrosis. In analyzing the results obtained, the observed regularity in the calculation of FIB-4 was found to be a significant difference between the test values in patients with normal body weight and overweight ($p<0.01$), normal body weight and I-grade obesity was obtained ($p<0.01$), overweight and I degree obesity ($p<0.01$). Comparative characteristics of FibroTest indicators by subgroups are given in Figure 2.

The significance of the difference in FibroTest values in the subgroups relative to anthropometry in terms of BMI is given in Table 4.

Discussion

Therefore, a correlation analysis found a significant strong relationship between Fibrotest and FIB-4 ($r=0.81$, $p<0.05$). In addition, a significant relationship was found between the presence of excess body weight and the severity of fibrosis ($r=0.79$, $p<0.05$). Thus, analyzing the calculations, we can conclude that for the dynamic monitoring of patients with liver fibrosis, it is advisable to perform both methods, as they are informative and complementary.

Analyzing the profibrogenic cytokine TGF-β1, it was noted that the lowest value was 6123 ng/ml, the highest level was 18756 ng/ml, with the average value for all examined patients was 13787±4563.3. It was found to increase significantly with increasing stage of fibrosis and BMI with maximum values in the group of patients with toxic liver disease and BMI with a value of 30 34.9 kg/m^2 ($p<0.05$). With the progression of liver fibrosis, the concentration of TGF-β1 ($r=0.80$, $p<0.05$) in

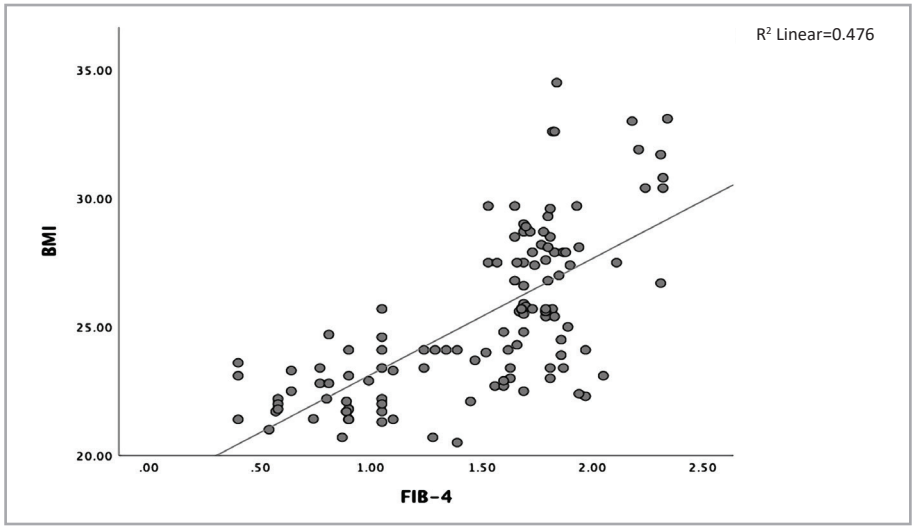

Figure 1: Dependence of Fib-4 level on BMI in patients.

Table 3: Indicators of FIB-4 Index in Subgroups (M±m).

Group	FIB-4		p
	Patients without signs of toxic liver disease	Patients with toxic liver disease	
BMI 18.5–24.9 kg/m²	0.9±0.05	1.43±0.04	<0.01*
BMI 25–29.9 kg/m²	1.46±0.01	1.84±0.06	<0.01*
BMI 30–34.9 kg/m²	-	2.17±0.04	<0.01*

Note: Significance of the difference according to the Kraskel-Wallis criterion: * – p<0.01.

the blood increases, which confirms the role of TGF-β1 in the activation of liver stellate cells and stimulation of collagen and other extracellular matrix synthesis and is likely a marker of the progression of fibrosing reactions (Table 5).

Therefore, analyzing the BMI, direct correlations with TGF-β1 (r=0.71, p<0.05). There was a significant relationship between the level of direct (TGF-β1) and indirect (ALT and AST) markers of fibrosis. Respectively, TGF-β1 with ALT level (r=0.63, p<0.05) and with AST level (r=0.68 p<0.05).

In addition, direct strong correlations of TGF-β1 with the stage of liver fibrosis according to the fibrotest (r=0.88, p<0.05) with FIB-4 index (r=0.79, p<0.05).

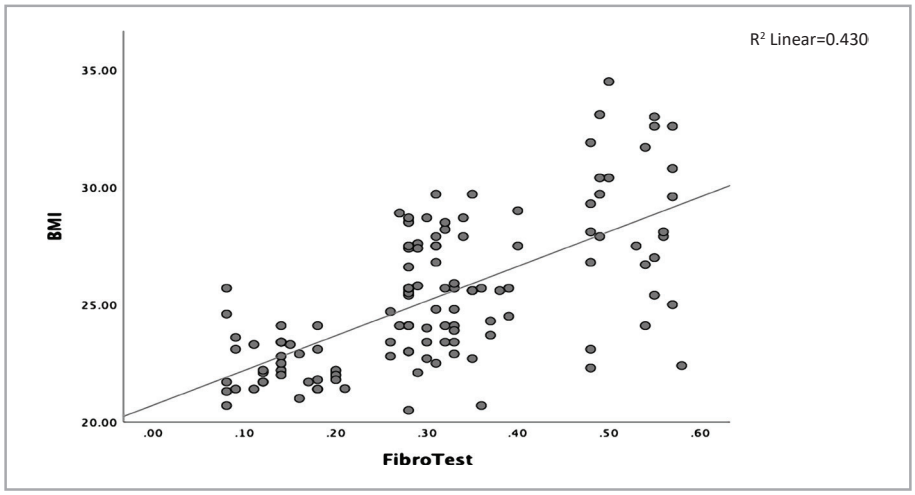

Figure 2: Dependence of FibroTest level on BMI in patients.

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Table 4: Indicators of the Fibrotest Index in Subgroups (M±m).

Group	Fibrotest		
	Patients without signs of toxic liver disease	Patients with toxic liver disease	p
BMI 18.5–24.9 kg/m²	0.12±0.01	0.27±0.01	<0.01*
BMI 25–29.9 kg/m²	0.28±0.05	0.39±0.02	<0.01*
BMI 30–34.9 kg/m²	0.38±0.03	0.51±0.02	0.01*

Note: Significance of the difference according to the Kraskel-Wallis criterion: * – p<0.01.

Table 5: Comparative Characteristics of TGF-β1 (M±m).

Group	TGF-β1, ng/ml		
	Patients without signs of toxic liver disease	Patients with toxic liver disease	p
BMI 18.5–24.9 kg/m²	6045.56±878.9	11233±757.5	0.016*
BMI 25–29.9 kg/m²	12910.70±600.1	13994.53±341.9	0.006*
BMI 30–34.9 kg/m²	18394.20±574.7	20744.502±271.8	0.121

Note: Significance of the difference according to the Kruskal-Wallis criterion: * – p<0.01.

Conclusions

FIB-4 and Fibrotest may be sensitive and reliable methods for liver fibrosis screening in patients after cancer chemotherapy. The presence of excess body weight correlates with the severity of liver fibrosis (r=0.79) and may be an important factor in predicting the development of severe fibrosis in patients after long-term breast cancer treatment. The level of profibrogenic cytokine TGF-β1 correlates with the severity of liver fibrosis with fibrotest (r=0.88) and FIB-4 index (r=0.79) and may be an additional predictor of fibrosis progression.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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