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Nonalcoholic fatty liver disease in patients with morbid obesity

Niealkoholowa stłuszczeniowa choroba wątroby w otyłości olbrzymiej

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S u m m a r y

Introduction. Accumulation of fat in obese individuals can impact hepatocytes and contributes to the inflammatory process, which in extreme cases results in fibrosis, cirrhosis, and failure of the liver.

Aim. The aim of this study was to assess the prevalence of nonalcoholic fatty liver disease (NAFLD), biochemical abnormalities accompanying this pathology, as well as the development of diagnostic method allowing the prediction of NAFLD or nonalcoholic steatohepatitis (NASH) among pathologically obese patients.

Material and methods. We retrospectively analyzed blood chemistry and liver biopsies obtained during surgery from 144 bariatric surgery patients. We also included 54 patients with evaluated blood levels of pro-inflammatory cytokines and their receptors.

Results. Out of 144 liver biopsies the 23% were normal, and isolated fibrosis was found in 5%, NAFLD was found in 72% of biopsies (where the isolated steatosis – NAS occurred in 28% and NASH – in 44%). Comparison of the data between the group with normal histopathology and with NAS showed that the second group had a significantly higher ALT and triglyceride levels but lower HDL. Comparison of subjects with normal liver and with NASH, we found that the first group had the lower concentration of ALT and GGTP, as well as lower levels of serum triglycerides. The average concentration of ALT in NASH group exceeded the normal limits. In assessing the concentrations of cytokines and their receptors we were able to develop formulas, which allow prediction of the NAFLD and NASH occurrence in a group of morbidly obese patients.

Conclusions. NAFLD occurs in most patients awaiting bariatric surgery. Elevation of ALT and AST activity, elevated concentrations of TNF-α and leptin and lower adiponectin levels in pathologically obese people are risk factors for NAFLD. Logistic regression model that applies above parameters allows prediction of the occurrence of obesity-related histological changes in the liver, without need for performing the liver biopsy.

Key words: bariatric surgery, fatty liver disease, cytokines, liver inflammation

S t r e s z c z e n i e

Cel pracy. Celem pracy była ocena częstości występowania niealkoholowej stłuszczeniowej patologii wątroby (NAFLD), zaburzeń biochemicznych towarzyszących tej patologii, jak również opracowanie sposobu pozwalającego na prognozowanie wystąpienia NAFLD lub niealkoholowego zapalenia wątroby (NASH) wśród chorych patologicznie otyłych.

Materiał i metody. Analizie retrospektywnej poddano badania biochemiczne krwi i wycinki wątroby uzyskane w trakcie zabiegu bariarycznego od 144 chorych. Badaniem prospektywnym objęto 54 chorych, u których dodatkowo oceniano stężenia cytokin prozapalnych i ich receptorów.

Wyniki. W 144 wycinkach wątroby badanie było prawidłowe w 23%, izolowane włóknienie stwierdzono w 5%, stłuszczeniową patologię wątroby aż w 72% (gdzie izolowane stłuszczenie – NAS stanowiło 28% i NASH – 44%). Analiza danych pomiędzy grupą z prawidłowym badaniem histopatologicznym i NAS wykazała, że w tej drugiej notuje się znamiennie wyższy poziom ALT (26,6 ± 11,3 vs 36,3 ± 18,3; $P < 0,05$) i trójglicerydów (112,5 ± 49 vs 164,1 ± 93,2 ; $P < 0,05$) natomiast niższy HDL (54,5 ± 13,9 vs 45,8 ± 11,7; $P < 0,04$). Porównując osoby z prawidłową wątrobą i NASH i stwierdzono odpowiednio w pierwszej grupie niższe stężenie ALT (26,6 ± 11,3 vs 56,6 ± 28,4; $P < 0,002$), niższe stężenie GGTP (9,3 ± 1,5 vs 36,0 ± 11,1; $P < 0,02$), jak również niższy poziom trójglicerydów w surowicy (112,5 ± 49 vs 180,6 ± 89,3; $P < 0,02$). Poziom HDL był znamiennie wyższy w grupie NASH (55,4 ± 13,9 vs 46,2 ± 10; $P < 0,02$). Średnie stężenie ALT w grupie

NASH przekraczało normę. Oceniając wyniki badań biochemicznych, stężeń cytokin i ich receptorów opracowano schematy pozwalające prognozować wystąpienia NAFLD i NASH w grupie patologicznie otyłych pacjentów.

Wnioski. U większości chorych zakwalifikowanych do zabiegu chirurgicznego leczenia otyłości występuje NAFLD. Zwiększenie aktywności ALT i AST, podwyższenie stężeń TNF-α i leptyny oraz obniżenie stężenia adiponektyny u osób patologicznie otyłych towarzyszą NAFLD. Opracowany model regresji logistycznej, pozwala na przewidzenie wystąpienia związanych z otyłością zmian histologicznych w wątrobie, bez konieczności wykonywania biopsji tego narządu.

Słowa kluczowe: chirurgia bariatryczna, stłuszczeniowa choroba wątroby, cytokiny, zapalenie wątroby

INTRODUCTION

Accumulation of fat in the organism affects almost all organs, and when it occurs in liver cells it contributes to the inflammatory process, which in extreme cases result in fibrosis, cirrhosis, and failure of the liver (1). Despite the fact that fatty liver is only the morphological feature, it presence correlates with diseases and metabolic disorders. The process of liver deterioration, which eventually results in liver failure is slow and for a long time asymptomatic. Clinical differentiation between simple steatosis and that with inflammatory reaction is impossible if one relies solely on biochemical analysis. The only method that allows for certain diagnosis is histopathological examination of the tissue, which, due to the risk of complications is performed very rarely. So far nonsurgical attempts to identify fatty liver disease, did not bring the expected results, mainly because the commonly used imaging techniques are non-specific and not sensitive enough to allow the assessment of the disease severity (2, 3).

Increasing hepatic lipid resources, increase the hepatocellular damage, through the oxidative stress of inflammation and proinflammatory cytokine activity. Not without significance is the phenomenon of insulin resistance found in obesity. Metabolic activity of adipose tissue is controlled by the endocrine system and its downstream components such as insulin, sex hormones and adrenal catecholamines.The morphological changes associated with steatosis of liver, not related to alcohol abuse, are called nonalcoholic fatty liver disease (NAFLD). Course of NAFLD, initially mild, is an insidious, contributing to the creation, in every fifth patient, a nonalcoholic steatohepatitis (NASH), which may progress in 19-33% of cases, into cirrhosis. The next step of the disease, launched by a simple accumulation of fat can progress from cirrhosis to the hepatocellular carcinoma (HCC) (4, 5). Incidence risk of NASH increases with the enlargement of body weight, from 7% in the general population to 80% in obese population (6-8).

Search for the causes of NAFLD highlighted its relationship with insulin resistance. Insulin alters lipid metabolism by increasing lipolysis in peripheral tissues, increasing the synthesis of triglycerides and severity of hepatic uptake of free fatty acids (9, 10). In the first phase of insulin resistance, the hyperinsulinemia, in combination with low levels of adiponectin and elevated TNF-α and leptin causes fatty degeneration leading to a second stage, the steatotic liver, which is more prone to hepatocyte injury. The oxidative stress, which affects lipid peroxidation and activates inflammatory cytokines that cause NASH, and the activation of fibrosis promoters ultimately lead to liver fibrosis and cirrhosis (11, 12).

The diagnosis of NAFLD is difficult. In half of the cases it is asymptomatic, sometimes it is accompanied by excessive fatigue, and nonspecific abdominal pain. In 75% of cases the physical examination shows hepatomegaly, splenomegaly and also less common disorders and symptoms of portal hypertension. Biochemical analysis of peripheral blood serum shows moderately elevated transaminase levels, but in contrast to the alcohol related fatty degeneration, the ratio of AST/ALT (de Ritis ratio) is usually less than 1 (13). The imaging tests can help in diagnosis, however, because of their low sensitivity and specificity they are not able to diagnose an early stage of the disease. They may only indicate a suspicion of the significant fibrosis and evaluate portal hypertension. They may, however, be useful when applied for the monitoring of the treatment. A very important role in the diagnosis of liver disease in obese patients plays a past medical history, allowing the identification of patients exposed to toxic etiology of the liver disease. NAFLD should not be identified in patients that consumed pure alcohol in an amount more than 40 g per week for men and 20 g for women (14).

In the presence of risk factors (such as obesity, type 2 diabetes, hyperlipidemia, and hypertension), and exclusion of other causes that may be responsible for an average increase of transaminases (such as hepatotropic virus infection, autoimmune hepatitis, hemochromatosis, α1-antitrypsin deficiency, galactosemia, tyrosinemia, Wilson's disease or inflammatory bowel disease) diagnosis of NAFLD is reliable only after histopathological evaluation (15). Only this examination allows to determine the degree of fatty degeneration, the presence of inflammatory infiltrates with foci of necrosis or advanced fibrosis. However, histopathological examination alone, excluding the medical history, not allows to distinguish NAFLD pathology from the damage caused by alcohol consumption (16-18).

Complexity of NAFLD prevention does not allow defining commonly acceptable treatment guidelines. The NAFLD treatment comprises of weight loss and the compensation of dyslipidemia and hyperglycemia (19).

Weight reduction in obese person results in an improvement in the metabolic, endocrine and psycho-

logical condition. In case of failure of conservative treatments for obesity, such as dietary therapy, lifestyle changes, psychotherapy and pharmacotherapy, a surgical treatment should be considered. Negative energy balance obtained after surgery is due to the reduced amounts of food eaten at single meal, and the effect of malabsorption. Surgery usually provides a much larger and longer-lasting weight loss than the conservative treatment.

MATERIAL AND METHODS

The data obtained from the medical records of patients undergoing bariatric surgery in the Division and the Department of General Surgery and Transplantology between 2001 and 2009 were retrospectively analyzed. The sera (obtained at least 24 hours before surgery) of these patients were analyzed for lipid content, markers of liver function and the level of high sensitive C-reacitve protein (hsCRP). During the procedure, a routine wedge biopsies of liver were also taken.

The histopathological evaluation data of the liver wedge biopsy were collected from 165 people. The total 21 patients were excluded from the study: 7 patients because of the symptoms indicating prior infection with hepatitis B virus (HBsAg positive or the presence of anti-HBc), 2 patients with hepatitis C virus (presence of anti-HCV antibodies in the serum) and 12 patients with uncertain history of alcohol abuse. Overall assessment of the liver biopsy was performed in 144 patients. In this group, the average age was 39.9 years, 79.2% were women, women were statistically younger than men (38.9 vs 43.6 years, $P < 0.02$). Mean BMI was 47.1 kg/m^2 and not statistically different between the two sexes.

The plasma from a group of prospective patients was analyzed using ELISA, for the level of adiponectin, leptin, interleukin-6 (IL-6), soluble receptor for IL-6 (sIL-6 R), an hsCRP, tumor necrosis factor α (TNF-α) and soluble receptors for TNF-α (sTNF RI and sTNF RII). Tests were performed immediately before surgery and 12 months later. The people who have had an uncertain history of alcohol abuse (more than 40 g of pure alcohol per week for men and 20 g for women) and those with a history of features of viral hepatitis were excluded from the analysis. A total of 54 patients, at the age of 39.9 years (21.1-58.7), were enrolled. 70.4% of all patients were women. Weight and BMI did not differ between the genders.

RESULTS

Out of total 144 liver biopsies taken from morbidly obese, the normal results were present in only 23% of biopsies. Isolated fibrosis was found in 5%, and fatty liver disease in 72% (which represented 28% of the NAS and 44% of NASH).

Anthropometric parameters (weight, age, sex) and biochemical test of serum were assessed in two groups of patients: with no liver pathology, and with abnormal picture in histopathological examination. We found that the group without liver pathology was younger (37.1 ± 12.2 vs. 40.7 ± 8.6 years, $p < 0.05$), had a lower concentration of alanine transaminase (25.9 ± 11.3 vs 47 , 9 ± 23.5 IU/L, $P < 0.006$), aspartate transaminase (26.6 ± 17.9 vs 31.6 ± 17,9 IU/L; $P < 0.05$), gamma-glutamyl transferase (9.3 ± 1.5 vs 31 ± 14.1 IU/L; $P < 0.04$), triglycerides (112.5 ± 48 vs 171.6 ± 81.8 mg/dL; $P < 0.03$) and higher HDL cholesterol (54.5 ± 13.9 vs 46.3 ± 10.7 mg/dL; $P < 0.01$).

In patients with abnormal liver histology mean ALT levels exceeded normal limits.

Mean body weight and BMI did not differ between the groups. Gender also had no effect on the occurrence of the liver pathology.

Comparison of the data between the group with normal histopathology and the NAS showed that the second group had a significantly higher level of ALT (26.6 ± 11.3 vs 36.3 ± 18.3 IU/L; $P < 0.05$) and triglycerides (112, 5 ± 49 vs 164.1 ± 93.2 mg/dL; $P < 0.05$) but lower HDL (54.5 ± 13.9 vs 45.8 ± 11.7 mg/dL; $P < 0.04$).

Similarly, were analyzed a group of NASH and normal histopathological outcome. The group without pathology had significantly lower weight (128.2 ± 21.5 kg vs. 140 ± 24.2 kg, $P < 0.02$), lower BMI (45.3 ± 7.3 kg/m^2 vs 48, 6 ± 6.1 kg/m^2, $P < 0.03$), lower levels of ALT (26.6 ± 11.3 vs 56.6 ± 28.4 IU/L; $P < 0.002$), lower levels of GGT (9.3 ± 1.5 vs. 36.0 ± 11.1 IU/L; $P < 0.02$), as well as lower levels of triglycerides (112.5 ± 49 vs 180.6 ± 89.3 mg/dL; $P < 0.02$). HDL cholesterol was significantly higher in this group (55.4 ± 13.9 vs 46.2 ± 10 mg/dL; $P < 0.02$). The average level of ALT in NASH group exceeded normal limits.

Comparison between the group of NASH and NAS showed statistical differences only in ALT (56.6 ± 28.5 vs 36.3 ± 18.3 IU/L; $P < 0.012$) and AST (35.9 ± 20.5 vs 26.1 ± 10.4 IU/L; $P < 0.03$) levels.

Evaluation of the risk of isolated fibrosis showed no correlation between specific pathology (NASH, NAS) and the normal picture of the liver.

Using Cox proportional hazard model we analyzed the impact of the presence of comorbidities of the obesity in the study group, such as type 2 diabetes, glucose intolerance, hypertension, obstructive sleep apnea and dyslipidemia on the NAFLD and we found an increased risk of NASH only in patients with sleep apnea ($\chi^2 = 9.040836$ $p = 0.0109$).

In the prospective group, prior to surgery, we tested the levels of: adiponectin, leptin, interleukin-6 (IL-6), soluble receptor for IL-6 (sIL-6 R), hsCRP, tumor necrosis factor α (TNF-α) and soluble receptors for TNF-α (sTNF RI and sTNF RII). In this group NASH was diagnosed in 32 and NAS in 14 cases. Only 8 patients had no liver pathology.

We found that the distribution of specific liver pathology was equal for both genders, has not been influenced by age, excess weight, BMI or fat mass. NASH group had a statistically higher percentage of fat in relation to the NAS group (49.4 ± 5.9% vs 44.4 ± 7.5%, $P < 0.04$).

Analyzis of the correlation matrix showed that the level of leptin was significantly higher (48.75 vs. 33.04 ng/mL; P = 0.037), while sTNFRII was lower in females than in men (3.04 v. 3.73 ng/mL; P < 0.003).

Comparison of the model of body fat distribution (visceral or gynoid) demonstrated no effect on the concentration of each protein in the serum.

Analyzis of the levels of cytokines and peripheral blood biochemical results in relation to the pathology of the liver found no statistical significance (tab. 1).

PREDICTION OF THE OCCURRENCE OF NONALCOHOLIC FATTY LIVER DISEASE

Using single variables shown in table 1 to identify patients with non-hazardous liver, requiring further proceedings (NASH or NAS) we employed logistic regression, for the indication of the sensitivity and specificity (tab. 2).

We established the optimal logistic regression model to estimate the risk of histological changes in the liver, including leptin, adiponectin and ALT activity. Details of the regression model are shown in table 3 (P < 0.0003).

Probability of histological changes of liver were calculated using all three variables and the formula:

$$P = \frac{e^z}{1 + e^z}$$

e – base of natural logarithms;
Z = 2.378-0.072 x (leptin) + 0.281 x (adiponectin) – 0.0778 x (ALT)

The calculated P-value less than 0.5 points to the possibility of NASH or NAS presentation at the liver histology. The P value above 0.5 indicates normal liver structure.

This model has a sensitivity of 95% and a specificity of 66.7%, with 90% of correctly classified cases.

PREDICTION OF NONALCOHOLIC STEATOHEPATITIS

Using a single variable and logistic regression account to identify the non-hazardous NASH patients we achieved sensitivity and specificity results presented in table 4.

Table 1. Concentration of cytokines and peripheral blood chemistry in relation to the pathology of the liver.

	Abnormal biopsy (n = 46)	Normal biopsy (n = 8)	P <	NASH (n = 32)	Non-NASH (n = 22)	P <
IL6	2.63 ± 1.65	1.44 ± 1.24	0.05	5.42 ± 4.9	2.53 ± 1.9	NS
TNFα	2.35 ± 0.5	2.03 ± 0.58	NS	2.44 ± 0.58	2.09 ± 0.35	0.02
Leptin	49.1 ± 22	26.3 ± 16.5	0.006	53.1 ± 23.7	33.9 ± 15.7	0.002
Adiponectin	4.65 ± 2.31	6.49 ± 2.44	0.04	4.33 ± 1.83	5.87 ± 2.88	0.02
AST activity	32.8 ± 13.3	23.3 ± 5.84	0.05	35.7 ± 13.2	24 ± 8.3	0.001
ALT activity	47.7 ± 22.9	26.6 ± 9.31	0.01	51.4 ± 23.1	32.6 ± 16.5	0.003
Total cholesterol	208.5 ± 43.5	153.5 ± 18.6	0.02	219.3 ± 44.7	174.3 ± 28.5	0.002
Triglycerides	163.6 ± 72.8	76.8 ± 42.3	0.03	171.9 ± 82.6	124.4 ± 48.4	NS

Note: abnormal biopsy = NAS or NASH

Table 2. The sensitivity and specificity of the variables used in the diagnosis of liver diseases associated with obesity.

	IL6	Leptin	Adiponectin	AST	ALT	Cholesterol	Triglycerides
The odds ratio	0.499	0.92	1.35	0.92	0.90	0.97	0.96
P <	0.03	0.002	0.05	0.03	0.002	0.02	0.005
Sensitivity	1	0.98	1	1	0.95	0.94	1
Specificity	0	0.22	0.12	0	0.22	0	0.5
% of correct	78.9	85.2	79.2	81.6	82	84.2	82.1

Table 3. Logistic regression model to estimate the risk of histological changes in the liver associated with obesity.

	Leptin	Adiponectin	ALT	Independent part (B)
Evaluation of parameter (Z)	-0.072	0.281	-0.0778	2.378

Table 4. The sensitivity and specificity of the variables used in the diagnosis of NASH.

	TNFα	Leptin	Adiponectin	AST	ALT	Cholesterol
The odds ratio	0.213	0.949	1.329	0.906	0.944	0.9735
P <	0.02	0.0008	0.02	0.0006	0.002	0.002
Sensitivity	0.78	0.78	0.844	0.73	0.80	0.833
Specificity	0.409	0.59	0.409	0.70	0.60	0.714
% of correct	63	70.4	66.7	72	72	78.95

The optimal regression model ($P < 0.00001$) is shown in table 5.

The likelihood of NASH of liver was calculated using all 4 variables using the formula:

$$P = \frac{e^z}{1 + e^z}$$

e – base of natural logarithms;
Z = 7.593-1.501 x (concentration of TNF) – 0.061 x (leptin) + 0.331 x (adiponectin) – 0.127 x (AST activity)

If the calculated value of P was less than 0.5, the result pointed to possibility of NASH. P values grater than 0.5 indicate the absence of hepatitis.

Sensitivity estimation was 90%, specificity 75%, with the 84% cases correctly classified.

We found that the weight loss was better in people who show no evidence of liver pathology during the operation. Mean% EWL in this group was 45.11% ± 29.80%, while for those with NAFLD this ratio was 29.92% ± 15.69%, $P < 0.023$ (for NASH and NAS respectively 30.49% ± 17.23%, $P < 0.04$ and 28.01% ± 11.22%, $P < 0.04$). Twelve months after surgery the group without liver pathology had significant reduction of fat mass (43.82 ± 14.47 kg) compared to the group with NAFLD – 33.92 ± 11.27 kg (NASH and the NAS respectively 34.01 ± 11.66 kg; $P < 0.04$ and 33.9 ± 10.28 kg, $P < 0.03$) (tab. 6).

The equalization of concentration of all tested cytokines and lower levels of both soluble receptors for TNF-α 12 months after surgery are shown in table 7.

DISCUSSION

Numerous studies confirm the beneficial effect of surgical treatment for obesity-related diseases. Scandinavian prospective study shows that among 845 patients treated surgically and 845 of the matched control group the incidence of hypertension, diabetes and lipid disorders was, 24 months after surgery, significantly lower in the surgical group (20). In the 8 years after surgery follow-up, the risk of diabetes decreased, while there was no effect on reducing the risk of hypertension (21, 22).

In our study, during the surgery, according to the generally prevailing views on the desirability of a liver biopsy in patients operated for obesity, we took wedge biopsy from the segment IVa of the liver, immediately after opening the abdominal cavity.

Tissue material was obtained from 165 individuals. We excluded 21 patients due to: features of prior in-

Table 5. Logistic regression model to estimate the risk of NASH.

	TNFα	Leptin	Adiponectin	AST	Independent part (B)
Evaluation of parameter (Z)	-1.501	-0.061	0.331	-0.127	7.593

Table 6. Changes in some anthropometric parameters observed 12 months after surgery in group NAFLD (NASH and NAS) compared to the group without liver pathology.

	NORM mean ± standard deviation	NAFLD mean ± standard deviation; P	NASH mean ± standard deviation; P	NAS mean ± standard deviation; P
Δ body mass (kg)	53.80 ± 17.52	42.71 ± 11.99; $P < 0.023$	42.24 ± 12.11; $P < 0.01$	43.68 ± 11.64; NS
Δ BMI (kg/m^2)	18.27 ± 5.20	15.25 ± 4.77; NS	15.24 ± 4.62; NS	15.12 ± 5.13; NS
Δ Fat% (%)	19.32 ± 4.15	15.98 ± 6.04; NS	15.57 ± 5.70; NS	17.34 ± 6.79; NS
Δ FAT (kg)	43.82 ± 14.47	33.92 ± 11.27; $P < 0.023$	34.01 ± 11.66; $P < 0.04$	33.90 ± 10.28; $P < 0.03$
Δ FFM (kg)	9.98 ± 6.04	8.55 ± 6.79; NS	7.91 ± 5.05; NS	9.76 ± 6.00; NS
%BMIL (%)	37.62 ± 4.96	32.71 ± 7.53; NS	31.42 ± 6.79; $P < 0.031$	33.41 ± 9.01; NS
%EBMIL (%)	82.08 ± 8.48	73.24 ± 15.96; NS	72.43 ± 16.24; NS	76.14 ± 15.48; NS
%EWL (%)	45.11 ± 29.80	29.92 ± 15.69; $P < 0.023$	30.49 ± 17.23; $P < 0.04$	28.01 ± 11.22; $P < 0.04$

Table 7. Concentration of cytokines and levels of soluble receptors for TNF-α 12 months after surgery in group NAFLD (NASH and NAS) compared to the group without liver pathology.

	NORM mean ± standard deviation	NAFLD mean ± standard deviation; P	NASH mean ± standard deviation; P	NAS mean ± standard deviation; P
IL-6 (pg/ml)	0.66 ± 0.59	0.75 ± 0.63; NS	0.75 ± 0.68; NS	0.73 ± 0.54; NS
IL-6sR (ng/ml)	46.19 ± 8.64	51.15 ± 11.49; NS	51.19 ± 11.24; NS	49.85 ± 12.86; NS
leptin (pg/ml)	12.98 ± 7.44	16.14 ± 10.04; NS	17.75 ± 9.93; NS	11.98 ± 9.22; NS
TNF-α (pg/ml)	2.48 ± 0.88	2.15 ± 0.30; NS	2.17 ± 0.31; NS	2.04 ± 0.25; NS
sTNF RI (ng/ml)	1.09 ± 0.16	1.46 ± 0.27; $P < 0.0002$	1.43 ± 0.29; $P < 0.004$	1.58 ± 0.23; $P < 0.0004$
sTNF RII (ng/ml)	2.27 ± 0.35	2.79 ± 0.53; $P < 0.01$	2.75 ± 0.59; $P < 0.04$	2.86 ± 0.38; $P < 0.002$
adiponectin (μg/ml)	15.41 ± 7.96	10.99 ± 6.62; NS	10.91 ± 6.73; NS	11.12 ± 6.36; NS
hsCRP (mg/l)	4.62 ± 2.23	4.86 ± 3.89; NS	5.62 ± 4.46; NS	3.56 ± 1.70; NS

fection with hepatitis B or C and uncertain history of alcohol abuse. The normal picture of liver was identified only in 23% of patients. These people were much younger than patients who had liver changes. In addition, they had statistically significant lower levels of ALT, AST, GGT and triglycerides. People with NAS, NASH or isolated fibrosis, representing respectively 28, 44 and 5% of all patients had a significantly lower mean HDL cholesterol levels. In addition, we analyzed differences occurring in biochemical findings between patients with diagnosed steatosis, inflammation and isolated fibrosis compared and patients with normal liver. The NAS and NASH groups showed significantly higher levels of ALT and triglycerides, and lower HDL. Additionally, the group without liver pathology had significantly lower body weight and BMI. In groups of NASH and NAS had statistically significant differences in transaminase activity – the higher values of ALTs and ASTs in the group with inflammation.

Our assessment of the risk of isolated fibrosis, showed no statistically significant differences between patients with diagnosed fibrosis and patients with other liver pathologies (NASH, NAS) and unchanged picture of the liver.

A meta-analysis of Machado et al. on 1620 patients with morbid obesity, the fatty liver was identified in 91% of cases (85-98%), and NASH in 37% (24-98%). Cirrhosis was diagnosed in 1.7% of patients (1-7%). In this group of patients there was no correlation between NASH, age or BMI (23).

The presence of elevated levels of transaminases, triglycerides and reduced HDL cholesterol in obese patients who in biopsy presented features of nonalcoholic fatty liver disease, is described in a number of original studies (24-26).

A separate group of studies, are these, trying to identify the factors that uniquely allow recognition of NAFLD, without the need for biopsy (27-29).

Investigators seek to identify markers allowing to identify with a high probability the subjects who would need histological examination of liver, which is so far the only diagnostic test that allows for reliable diagnosis and assessment of the progress of treatment of various forms of non-alcoholic fatty disease (30). It must be remember that despite the highest sensitivity and specificity of histopathological evaluation, this method does not allow to distinguish NFLD from the alcohol related fatty degeneration (16-18).

The role of elevated levels of transaminases, especially ALT, as a biomarker of liver pathology in obese patients has been described in several recent studies (26, 31).

Fracanzani et al. rated 458 liver biopsies and found elevated level of transaminase in 86% of patients diagnosed with NAFLD based on biopsy. In this group, those with normal ALT levels were statistically older and had a lower BMI. NASH was diagnosed in patients with normal or elevated levels of ALT, respectively, 59% and 74% ($P = 0.01$). The authors tried to determine the suitability of the ALT level for the prediction of the possible occurrence of NASH. They data showed an increase of the risk of 1.04-1.19, with each increase in the ALT level of 10 U/mL. Unfortunately, it turned out that the normal ALT values do not exclude the possibility of liver pathology in obese patients (32).

In our study we identified the factors predisposing to the occurrence of nonalcoholic fatty liver disease. In the prospective study we enrolled 54 patients, aged from 21.1 to 58.7 years (mean 39.9 years). In 59% of those we diagnosed NASH, 26% NAS, while 15% of patients had no liver pathology. Statistically, the frequency of occurrence of liver pathology (normal, NAS and NASH) were similar, irrespective of sex and age (respectively: 40.5 years, and 40.9years 39.2 years; NS), excess weight on the day of surgery (respectively: 79.2 kg, 64.2 kg and 68.7 kg; NS), BMI (respectively 48.4 kg/m^2, 44.2 kg/m^2 and 46.2 kg/m^2; NS), and the weight of the total body fat (respectively: 71.6 kg, 57.3 kg and 64.5 kg; NS). Patients with NASH had statistically significant higher percentage of body fat compared to patients with NAS (49.0% vs 44.4%).

From the sera collected prior to surgery, we measured the levels of adiponectin, leptin, interleukin-6 soluble receptor for IL-6, acute phase proteins, tumor necrosis factor TNF-α and soluble receptors for TNF-α.

Previous studies indicated that there is a correlation between the body fat distribution and concentration of specific cytokines in the serum (REF). However our present study show that there is no statistically significant difference between obesity type and specific protein concentration in the serum.

Analysis of the concentration of individual cytokines and their receptors and the biochemical study of peripheral blood in relation to the pathology of the liver, showed that patients with normal biopsy result had a significantly lower levels of IL-6, leptin, total cholesterol, triglycerides, and lower the activity of AST and ALT, when compared to the group with liver pathology group. However, adiponectin levels were significantly higher in this group. A similar distribution of individual proteins and enzymes has been reported previously (33-35).

Our study shows that the determination of the level of ALT and AST as well TNF-α, leptin and adiponectin, can be very useful for the prediction of the occurrence of liver pathology, without a need for biopsy.

Angulo et al., determined the risk of advanced liver fibrosis in patients with NAFLD. Using a simple scoring system based on routinely measured and readily available demographic, clinical and laboratory data, they tried to identify people without advanced fibrosis or process of this pathology in patients with NAFLD. 733 patients with biopsy-proven NAFLD were divided into 2 groups. The first ($n = 480$) was used to create, and the second ($n = 253$) to confirm the adopted scoring system. Routine demographic, clinical and laboratory variables were analyzed by multivariate modeling, allowing predicting the presence or absence of

advanced fibrosis. The following indicators of advanced liver fibrosis were assessed: age, blood glucose, body mass index, platelet count, albumin level, and the ratio of AST/ALT. Using a low threshold scale (-1.455), the researchers were able to determine the presence of advanced fibrosis with high accuracy (negative predictive values 93% and 88%, respectively, for the assessment and approval groups). In determining the appropriate high point threshold (0.676), they estimated advanced fibrosis with high accuracy (90% and 82%) in the estimation and validation group. These authors concluded that the introduction of this model would easily identify patients with NAFLD without advanced fibrosis (36).

In our study, we showed that people who did not have liver pathology at the time of surgery had more body weight loss (53.8 kg vs. 42.7 kg), more body fat mass reduction one year after surgery (43.8 kg vs. 33.9 kg).

The analysis of the changes in concentrations of specific proteins twelve months after surgery, showed statistically significant increase in adiponectin levels (respectively 4.96 μg/mLl vs 11.64 μg/mL; P = 0.000001), lower levels of interleukin-6 (4.24 pg/mL vs 0.73 pg/mL, p = 0.000036), leptin (45.27 ng/mL vs. 15.55 ng/mL, p = 0.000001), hsCRP (15.65 mg/L vs 4.59 mg/L, P = 0.0002) as well soluble receptors for TNF-α: sTNF RI (1.85 ng /mL vs 1.42 ng/mL, P = 0.000001) and sTNF RII (3.28 ng/mL vs 2.71 ng/mL, P = 0.000001). In contrast the TNF-α levels (2.3 pg/mL vs 2.2 pg/mL: NS) and the receptor for interleukin-6 (51.56 ng/mL vs. 50.51 ng/mL; NS) remained unchanged.

During the follow-up there was observed the equalization of concentrations of most cytokines among the different groups of patients (without liver pathology, NASH, NAS) with the exception of the level of soluble receptors for TNF-α, which in the 12 months after surgery was significantly lower in patients without liver pathology on the day of surgery (1.09 ng/mL vs 1.46 ng/mL for sTNF RI and 2.27 ng/mL vs 2.79 ng/mL for sTNF RII).

The observed changes may indicate a regression of inflammatory changes in the group of patients previously diagnosed with NAFLD.

CONCLUSIONS

1. Nonalcoholic fatty liver disease occurs in most patients.
2. Elevations of ALT and AST activity, elevated concentrations of TNF-α and leptin and lower adiponectin levels in pathologically obese people are risk factors for nonalcoholic fatty liver disease.
3. Logistic regression model that applies above parameters allows for the prediction of the occurrence of obesity-related histological changes in the liver, without performing a biopsy of the liver.

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