The Clinical Value of Soluble Cytokeratin-18 in Differentiating Simple Steatosis from Non Alcoholic Steatohepatitis

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ABSTRACT

Non alcoholic steatohepatitis (NASH) could be present in one third of non alcoholic fatty liver disease (NAFLD) cases and appears to have a higher likelihood of progression to cirrhosis. An increased risk of hepatocellular carcinoma and end-stage liver disease has been reported among patients with NASH. However, liver biopsy is an invasive procedure with unavoidable risks and limitations and it is not relevant to the choice of treatment. Therefore, the development of non invasive tests for assessing hepatic inflammation and fibrosis has become an active area of research. The present study aimed to investigate whether serum levels of two soluble forms of extracellular cytokeratin 18 (M30-antigen and M65-antigen) may differentiate NASH from simple fatty liver in patients with NAFLD. Fifty eight patients with suspected NAFLD were classified according to their liver histology into two groups (27 of NASH and 31 of simple steatosis), and 25 healthy age- and gender-matched volunteers were enrolled in the study. Clinical examination, anthropometric measurements, abdominal ultrasound and liver biopsy were done to all patients. Laboratory investigations which included lipid profile, liver function tests and fasting insulin level were done. Serum levels of two soluble forms of extracellular cytokeratin 18 (M30-antigen and M65-antigen) were measured. Serum levels of M30-antigen and M65-antigen were significantly higher in patients with definitive NASH compared to the group of simple steatosis and control group. At cut-off value >114.7 U/L of M30-antigen yielded an 81.5% sensitivity and a 93.5% specificity and M65-antigen at the cut-off level >254.5 U/L gave a sensitivity of 70.4% and specificity of 77.5% for the diagnosis of NASH. The level of each M30 and M65 antigens correlated positively with AST & ALT activities and histological features of NAFLD patients. In conclusion: these findings suggest that determination of CK-18 fragments in the blood could be used as a non invasive predictor of NASH and highlight the potential usefulness of that test as a noninvasive diagnostic means of determining histological disease severity in patients with NAFLD.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represents a group of conditions ranging from simple liver steatosis, usually asymptomatic, to nonalcoholic steatohepatitis (NASH), which is characterized by the presence of apoptosis/inflammation and fibrosis, and also by a progressive course, evolving to cryptogenic cirrhosis[(1)]. Although NAFLD typically follows a benign non progressive clinical course, NASH is a potentially serious condition, since as many as 25% of patients may progress to cirrhosis and experience complications of portal hypertension, liver failure and hepatocellular carcinoma [(2&3)].

At present, the available non-invasive tests to distinguish NASH from NAFLD include clinical signs and symptoms, routine laboratory and radiological imaging tests and combinations of clinical and blood test results [(4)].

The sensitivity of ultrasonography in detecting steatosis varies between 60% and 94%, and also varies depending on steatosis degree. Another difficulty consists of the impossibility of identifying the inflammatory changes of the hepatic parenchyma and to differentiate simple steatosis from steatohepatitis. It is also difficult to differentiate steatosis from liver fibrosis, because both of them have similar appearance on ultrasound[(5)]. Computed tomography and magnetic resonance imaging are other alternatives, but their use is limited because they are expensive and the information they provide is limited. Serological markers for fibrosis assessment are frequently used in Europe, in contrast with the United States where liver biopsy is preferred. Different tests have been used for evaluating fibrosis, such as the AST/ALT ratio and the aspartate / platelet ratio index (APRI) test, which assesses platelets and AST levels. The most important defect of these types of tests is their inability to distinguish between mild and moderate fibrosis, knowing that early detection of fibrosis is valuable for preventing disease progression [(6)].

The utility of these tests is limited in cases with advanced fibrosis and liver biopsy remains the only reliable way of diagnosing NASH and grading the severity of liver damage. However, it is obvious that an invasive liver biopsy is poorly suited as a diagnostic test in such a prevalent condition. There is, therefore, an urgent need to develop and validate a simple, reproducible, non-invasive test that accurately distinguishes NASH from NAFLD and determines the stage and grade of the disease[(7)].

Cytokeratin (CKs) represent a multigene family of cytoskeletal proteins, typically and abundantly present in epithelial cells, in which they form bundles of intermediate-sized filaments[(8&9)]. CKs are present in certain simple epithelial cells such as hepatocytes [(10&11)]. Cytokeratin 18 is a relatively new marker that derives from the caspase-3 pathway; however, to date, it has limited utility in clinical practice and is used only for research purposes [(1)].

Hepatocyte apoptosis is a prominent pathologic feature of

human NASH [12] and the magnitude of apoptosis present correlates with degree of liver damage and stage of fibrosis. Experimental studies suggest that uncontrolled hepatocyte apoptosis may be a central mechanism triggering liver fibrogenesis and fibrosis [13]. Cytokeratin-18 represents a marker of hepatocyte apoptosis, and its value as a potential biomarker for NASH is based on the observation that apoptosis is prominent in NASH and absent in simple steatosis [14].

Aim:

The objective of the present study was to validate the clinical value of determination of the CK-18 fragment levels in blood for NASH diagnosis and assessment of disease severity in a large cohort of well characterized NAFLD patients.

PATIENTS & METHODS

Fifty eight patients (32 males and 26 females), age ranged 34-57 years, with suspected NAFLD were enrolled in the study. They attended National liver Institute from January 2010 to June 2011. Additionally, 25 healthy age- and gender-matched volunteers were recruited. All controls were judged to be in good health, with normal results on liver function tests and confirmed as having normal liver by ultrasound. A written informed consent was obtained from all participants. The study protocol was reviewed and approved by the Ethics Committee of the National Liver Institute-Menofia University.

Exclusion criteria: Patients with viral hepatitis, hemochromatosis, Wilson's disease, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, biliary obstruction, alpha-1 antitrypsin deficiency, or malignancies were excluded from the present study. None of the subjects was using any medications including estrogens, amiodarone, steroids, tamoxifen, or herbal supplements.

All subjects in the study underwent the followings:

1- Physical and clinical examination
2- Anthropometric measurements: Body mass index (BMI) was calculated as weight (in kilograms) divided by height squared (meters squared). Waist circumference (at the nearest half centimeter) was measured at the midpoint between the lower rib margin and the iliac crest.
3- Abdominal ultrasound (US): Liver steatosis was assessed semi-quantitatively on a scale of 0 to 3: 0, absent; 1, mild; 2, moderate; and 3, severe.
4- Liver biopsy was done to all patients:
   Biopsies were fixated and embedded in paraffin blocks and stained with hematoxylin-eosin, Masson's trichrome. The samples were scored according to the NIDDK NASH Clinical Research Network scoring system[15]. Steatosis was scored from 0 to 3, with a four grades scoring system from S0 to S3: S0: no steatosis or less than 5%, S1: 5%-33%, S2: 33% - 66%, S3: > 66%. Lobular inflammation was graded as follows: stage 0, no foci; stage 1: < 2 foci per 200 × field; stage 2: 2-4 foci per 200 × field; stage 3: > 4 foci per 200× field. Fibrosis was staged as follows: stage 0: no

fibrosis; stage 1: perisinusoidal or periportal fibrosis; stage 2: perisinusoidal and portal/periportal fibrosis; stage 3: bridging fibrosis; stage 4: cirrhosis. The histological NASH score was defined as the sum of the scores for steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2); thus ranging from 0 to 8. Cases with scores of 0 to 2 were considered as having simple steatosis; on the other hand, cases with scores of 5 or greater were diagnosed as definitive NASH.

Patients with suspected NAFLD were classified according to their liver histology into two groups: definitive NASH (n = 27) and simple fatty liver (simple steatosis; n = 31).

5- Laboratory investigations:

Venous blood samples were obtained after a fasting night. Part of the sample was placed in heparinized tubes, and circulating leukocytes were isolated for subsequent studies. Another part of the blood sample was collected in pyrogen free plain tubes, then centrifuged and the resulting serum was divided into aliquots and kept at –70°C until assayed**.** An aliquot of serum was used to monitor lipid profile including: total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides, fasting blood sugar, and to perform routine laboratory examinations {serum albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase, total & direct bilirubin and total protein} were measured using COBAS Integra-400 autoanalyzer (Roche- Germany). The determination of fasting insulin level was done using Diagnostic System Laboratories incorporation kits {DSL-10-1600 ACTIVE® insulin, enzyme linked immunosorbent assay (ELISA) kit, Texas- USA}. It is an enzymatic amplified one step sandwich type immunoassay. The minimum detection limit was 0.26 µIU/ml; the intra- and inter-assay coefficient of variations was 2.6% and 6.2% respectively. Standards, controls and samples were incubated with (HRP) labeled anti-insulin antibody in microtitration wells which were coated with another anti-insulin antibody, the assay was performed according to the manufacturer's instructions[16]. The degree of insulin resistance was calculated from the homeostasis model assessment (HOMA). The HOMA index was calculated by the formula [17]:

$$\frac{\text{Fasting plasma insulin (µIU/ml /ml) x fasting plasma glucose (mmol/L)}}{22.5}$$

HOMA index >3 is a criterion of insulin resistance [18].

6- Serum levels of M30-antigen and M65-antigen were determined by commercially available immunoassays TPS™ kit. It is an ELISA kit for the quantitative measurement of the soluble fragments of CK18 (M30 and M65) in serum provided by (IDL

Biotech, Peviva AB, Bromma, Sweden), it was done according to the manufacturer's instructions[19&20].

Statistical analysis

Descriptive statistics were computed for all variables. These include means and standard deviations. ANOVA test was used to compare parametric variables and Kruskal-Wallis test to non parametric variables between the three subject groups.. Spearman's correlation coefficients were used to assess associations between CK-18 levels and histological characteristics. To predict the presence of NASH with optimal sensitivity and specificity, Receiver Operating Characteristic (ROC) curve was used to estimate potential cut-off values of plasma CK-18 fragments. $P < 0.05$ was retained for statistical significance.

RESULTS

Table (1) showed the clinical, anthropometric and biochemical characteristic in each of the three studied groups (NASH group, simple steatosis group and control group).

Regarding liver ultrasonographic and histopathologic data, the comparison between both patient groups revealed that 15 patients (55%) in NASH group showed moderate degree of hepatosteatosis, while the majority of patients in simple steatosis (74.2%) showed mild degree as shown in table (2).

Analysis of the liver function tests in the three studied groups (table 3) revealed a significant difference between these groups regarding the mean serum activities of AST, ALT, GGT and alkaline phosphatase where the highest increase of these tests was found in the NASH group.

Table (4) showed a highly significant increase in the mean serum level of both M30-antigen and M65-antigen in the NASH group and simple steatosis group as compared to control group.

At cut-off value >114.7 IU/L; M30-antigen yielded a 81.5% sensitivity and a 93.5% specificity, M65-antigen at the cut-off level >254.5 IU/L gave a sensitivity of 70.4% and specificity of 77.5% for the differentiation of NASH from simple steatosis as reported in table (5).

Table (6) showed that in both patient groups (NAFLD patients), a significant positive correlation was observed between M30-antigen level and each of AST, ALT and simple steatosis with r=0.31, 0.29 and 0.37 respectively. While a significant positive correlation was observed between M65-antigen and each of AST & ALT only with r=0.37 and 0.32 respectively.

Table (1) Shows study group characteristics

Variables	NASH (n=27)	Simple steatosis (n=31)	Controls (n=25)	ANOVA test	p-value
Age (years)	42.3±7.5	39.4±4.2	36.8±3.2	6.15	>0.05
Gender (male)	15 (55.6%)	17 (54.8%)	15 (60%)	4.06	>0.05
BMI (kg/m²)	32.4±3.7	27.3±2.9	23.3±2.6	10.37	<0.05
Waist circumference (cm)	95.3± 7.2	88.3±5.1	84.2±4.5	9.09	<0.05
Systolic blood pressure (mmHg)	127±14	121±12	118±9	5.98	>0.05
Diastolic blood pressure (mmHg)	85±6	78±9	75±5	5.02	>0.05
Triglyceride (mg/dl)	157.4±18.5	126.7±22.7	96.7±15.3	12.11	<0.05
Total cholesterol (mg/dl)	216.2±22.1	185.2±16.7	155.2±16.5	23.27	<0.01
HDL (mg/dl)	31.4±5.2	35.7±3.7	39.4±4.6	5.11	>0.05
LDL (mg/dl)	123.3±10.9	118.1±17.6	42.1±5.1	18.47	<0.01
Fasting glucose (mg/dl)	95.6±10.4	90.6±7.2	83.5±9.3	8.91	<0.05
Fasting insulin (µIU/ml)	9.3±2.7	8.9±2.3	6.5±1.2	11.69	<0.05
HOMA index	3.6±1.52	2.9±1.22	2.5±0.74	14.23	<0.05

NASH: Nonalcoholic steatohepatitis BMI: body mass index
P>0.05 is no significant and p value <0.05 is significant.

Table (2) Liver utlrasonographic and histopathologic findings of NAFLD patients

Parameters	Description	NASH (n=27) No (%)	Simple steatosis (n=31) No (%)
US grade of hepato-steatosis:			
Mild	Scale 1	7 (25.9%)	23 (74.2%)
Moderate	Scale 2	15 (55.6%)	5 (16.1%)
Severe	Scale 3	5 (18.5%)	3 (9.7%)
Liver histology:			
Simple steatosis	<5% (score 0)	0%	0
	5-33% (score 1)	4 (14.8%)	29 (93.5%)
	33-66% (score 2)	11 (40.7%)	2 (6.5%)
	>66% (score 3)	12 (44.5%)	0
Lobular inflammation	No foci (score 0)	0	26 (83.9%)
	< 2 foci (score 1)	2 (7.4%)	5 (16.1%)
	2-4 (score 2)	19 (70.4%)	0
	>4 (score 3)	6 (22.2%)	0
Ballooning	None (score 0)	0	20 (64.5%)
	Few balloon cells (score 1)	4 (14.8%)	11 (35.5%)
	Prominent ballooning (score 2)	23 (85.2%)	0
Fibrosis stage	Grade 0	7 (25.9%)	31 (100%)
	Grade 1	11 (40.7%)	0
	Grade 2	6 (22.2%)	0
	Grade 3	3 (11.2%)	0
	Grade 4	0	0

NAFLD: Non-alcoholic fatty liver disease US: Ultrasound

Table (3) Comparison between patient groups and control group as regard liver function tests

Variables	NASH (n=27) Mean ±SD	Simple steatosis (n=31) Mean ±SD	Controls (n=25) Mean ±SD	Test of significance	p-value
AST (U/L)	43.6±11.5	28.7±6.3	20.5±7.4	19.2*	<0.05
ALT (U/L)	56.2±16.8	36.7±12.1	19.7±8.7	21.8*	<0.05
Alk. Ph. (U/L)	68.2±12.4	53.2±8.6	32.3±6.5	18.6	<0.05
GGT (U/L)	56.4±10.8	49.5±7.5	35.2±6.1	26.5*	<0.05
S.blirubin(mg/dl)	1.7±0.42	1.4±0.51	1.2±0.22	6.2	>0.05
T. proteins (g/dl)	7.6±0.8	7.1±1.4	7.4±1.2	7.8	>0.05
S. albumin (g/dl)	3.95±0.52	4.12±0.61	4.27±0.73	5.7	>0.05
Prothrombin con. %	78.1±7.4	80.2±9.2	92.4±3.5	6.9*	>0.05

* Kruskal-Wallis test was used.

P>0.05 is no significant and p value <0.05 is significant.

Table (4) Comparison between patients groups and controls as regard levels of cytokeratin 18

Variables	NASH (n=27)	Simple steatosis (n=31)	Controls (n=25)	Test of significance	p-value
M30-antigen (U/L)					
Range	128-396	43-106	27-81	39.3*	<0.001
Mean ±SD	259.3±131.6	72.7±29.4	52.7±29.5		
M65-antigen (U/L)					
Range	209-532	142-267	112-197	42.6*	<0.001
Mean ±SD	397.6±156.8	225.7±51.2	146.2±51.4		

* Kruskal-Wallis test was used. P<0.001 is highly significant.

Table (5): The sensitivity and specificity of cytokeratin 18 parameters in differentiating NASH from simple steatosis

Parameters	Cut-off (U/L)	Sensitivity	Specificity	PPV	NPV
M30-antigen	114.7	81.5%	93.5%	91.7%	85.3%
M65-antigen	254.5	70.4%	77.5%	71.2%	92.3%

PPV: Positive predictive value NPV: Negative predictive value

Table (6): Correlation between each M30-antigen and M65-antigen levels and prognostic parameters in NAFLD patients (n=58)

Parameters	**M30-antigen**		**M65-antigen**	
	r-value	p-value	r-value	p-value
AST (U/L)	0.31	<0.05	0.37	<0.05
ALT (U/L)	0.29	<0.05	0.32	<0.05
Simple Steatosis	0.37	<0.01	0.21	>0.05
Lobular inflammation	0.54	<0.001	0.17	>0.05
Ballooning	0.48	<0.001	0.11	>0.05
Liver fibrosis	0.51	<0.001	0.39	<0.05

DISCUSSION

Several investigators have tried to identify potential non-invasive markers for NASH diagnosis; however none of these markers have been externally validated[21,22&23]. Noninvasive panels of serological markers have been developed to evaluate the presence of steatosis and hepatic necroinflammation to avoid liver biopsy[24].

For that purpose the present study was designed to find the value of one of non invasive serum markers to predict NASH patients and to differentiate them from simple steatosis. Fifty eight patients (27 of NASH and 31 of simple steatosis), and 25 healthy age- and gender-matched volunteers were enrolled in the study. The caspase generated CK-18 fragments (M30-antigen and M65-antigen) were measured in patient's serum and compared with liver biopsy findings.

In the current study, serum levels of M30-antigen and M65-antigen were significantly higher in patients with definitive NASH compared to the group of simple steatosis and control group. Similar results were subsequently observed in an independent population of morbid obese patients undergoing bariatric surgery[25]. Our present results confirm and expand previous findings by those of **Yilmaz** et al.[26] and Feldstein et al.[7] on the potential clinical usefulness of different forms of CK18 (M30-antigen and M65-antigen) as biochemical assays to accurately distinguish definitive NASH from simple fatty liver.

Recent data also demonstrated that DNA from apoptotic hepatocytes acts as an important mediator of hepatic stellate cell activation[27]. Thus, non-invasive quantification of hepatocellular apoptosis represents a rational approach to assess the extent of liver damage and fibrosis present in the liver at a given time and also fibrogenesis and the risk for disease progression overtime. In hepatocytes, regardless of the triggering stimuli, the apoptotic process tends to converge at the level of the mitochondria resulting in permeabilization of the mitochondrial outer membrane and release of multiple proteins from the

mitochondrial intermembrane space into the cytosol[28&29]. The result of this process is the activation of the effector caspases (mainly caspase 3) which cleaves different substrates inside the cell including cytokeratin 18 (CK-18), the major intermediate filament protein in the liver, resulting in apoptosis [14&30].

Cytokeratin-18 represents a marker of hepatocyte apoptosis, and its value as a potential biomarker for NASH is based on the observation that apoptosis is prominent in NASH and absent in simple steatosis [31].

A report by **Wieckowska et al.**[32] had shown that measurement of serum M30-antigen levels may allow discrimination of definitive NASH patients from simple fatty liver with high sensitivity and specificity. However, the sample size in that study was small, and no attempt was made to study the concentrations of M-30 antigen in patients with possible NASH.

Diab et al.[25] showed that this marker accurate predicted NASH in an independent population of morbid obese subjects, while subsequently other groups have reported similar results [26&33]. Using this novel approach in a recent study, this study was able to demonstrate that determination of CK-18 fragments in the blood accurately identifies the presence of NASH and the severity of fibrosis on liver biopsy in adult patients with well-characterized NAFLD [14].

Bantel et al. [34] demonstrated that hepatitis C virus patients had elevated levels of blood caspase–generated CK-18 cleavage fragments compared with healthy controls. Caspase activity levels mirrored the degree of steatosis in these patients and correlated with fibrosis in a specific subset of subjects. **Wieckowska et al.**[32] were able to demonstrate that *in vivo* quantification of hepatocyte apoptosis accurately predicts NASH and they added that plasma caspase– generated CK-18 cleavage fragments were strikingly increased in patients with "definitive NASH" compared with those with "not NASH" and patients with suspected NAFLD but normal liver biopsy.

Moreover, caspase activity levels independently predicted the presence of NASH. Because of its high sensitivity, specificity, and positive and negative predictive value, this test has the potential to become an important instrument in clinical practice. In the present study, the appropriate cut-off values for serum M-30 antigen that distinguish between NASH and simple fatty liver was >114.7 U/L, it yielded a 81.5% sensitivity and a 93.5% specificity and M65-antigen at the cut-off level >254.5 U/L gave a sensitivity of 70.4% and specificity of 77.5%.

Feldstein et al.[7] reported that, the CK-18 test is able to detect the presence of NASH with a specificity of more than 90%, or to exclude the presence of NASH with sensitivity close to 80% by adopting different test thresholds. **Diab et al.**[25], recorded that at cut-off 275 U/L for CK-18, it gives a specificity of 100% and a sensitivity of 77%, for diagnosis of NASH. However, **Yilmaz et al.**[26], stated that , the cut-off values for serum M-30 antigen and M65-antigen were 121.60 U/L (sensitivity, 60.0%;

specificity, 97.4%) and 243.82 U/L (sensitivity, 68.9%; specificity, 81.6%), respectively.

In the current study, the level of M30-antigen correlated positively with AST & ALT activities and histological features of NAFLD patients. These changes were greater in those subjects with NASH and positively correlated with changes in transaminases, suggesting that measuring CK-18 fragment levels in the blood may be a useful test to monitor disease status. **Wieckowska et al. [32]** showed that cytokeratin 18 fragments were strikingly increased in the serum of patients with NASH and correlated with the presence of fibrosis. **Yilmaz et al. [26]** found that hepatic transaminases were significantly correlated with levels of both M-30 antigen (a marker of apoptosis) and M-65 antigen (a marker of necrosis). Also, **Diab et al.[25]** reported similar results.

Conclusion: In summary, the results of the present study suggest that non invasive monitoring of different forms of CK18 (M30-antigen and M65-antigen) in sera of patients with suspected NAFLD may represent a reliable tool to differentiate definitive NASH from simple fatty liver. Further validation studies in larger groups of patients are needed to confirm these findings.

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القيمة الاكلينيكية للسيتوكيراتين ١٨ الذائب في التفرقة بين التنكس الدهني البسيط من التنكس الدهنى الكبدى غير الكحولي

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يمثل التنكس الدهنى الكبدى غير الكحولى (ناش) الثلث من مرضى الكبد الدهني غير الكحولي. ويعتبر الاكثر احتمالية للتحول إلى تليف الكبد وزيادة مخاطر الاصابة بسرطان الخلايا الكبدية. وحيث ان اخذ عينة كبدية تؤدى الى الكثير من المخاطر التى لا يمكن تجنبها كما انها ليست ذات الصلة لاختيار العلاج. لذا ، فقد أصبح تطوير الاختبارات لتقييم التهاب الكبد وتليفه ضرورى فى الابحاث. يهدف هذا البحث الى دراسة ما إذا كانت مستويات المصل لاثنين من السيتوكيراتين ١٨ الذائب الموجود خارج الخلية (م٣٠انتيجين - م٦٥انتيجين) قد تفرق بين مرضى ناش من مرضى التنكس الدهني البسيط. وقد تم دراسة ثمانية و خمسين من مرضى الكبد الدهنى غير الكحولى من جامعة المنوفية وجامعة الازهر وقد تم تقسيمهم بحسب التحليل الهستولوجى لأنسجة الكبد إلى مجموعتين (المجموعة الاولى ٢٧ من مرضى يعانون من ناش والمجموعة الثانية ٣١ من مرضى يعانون من تنكس دهني بسيط) ومقارنتهم بخمسة و عشرون من الاصحاء متطابقين معهم فى السن والجنس. وقد أجري الفحص السريري ، وقياسات الجسم البشري ، الموجات فوق الصوتية على البطن والعينة الكبدية لجميع المرضى. وقد تم اجراء تحاليل معملية وتشمل نسبة الدهون ، وظائف الكبد وقياس نسبه الانسولين بالدم. كما تم قياس مستويات الدم لاثنين من السيتوكيراتين ١٨ الذائب الموجود خارج الخلية (م٣٠انتيجين - م٦٥انتيجين). وقد اثبتت التحليلات الاحصائية ان مستويات كلا من م٣٠انتيجين - م٦٥انتيجين أعلى بكثير في المرضى الذين يعانون من ناش بالمقارنة مع مجموعة مرضى التنكس الدهني البسيط ومجموعة الاصحاء. وقد وجدت علاقة ارتباط ايجابية ذات دلالة احصائية بين م٣٠انتيجين وكلا من انزيمات الكبد و التحليل الهستولوجى لانسجة الكبد فى مجموعتى المرضى. مما سبق يتضح ان نسبة السيتوكيراتين ١٨فى الدم قد تستخدم كمؤشر لحدوث مرض ناش. كما يجب التاكيد على الفائدة المحتملة لهذا الاختبار باعتباره وسيلة لمعرفة درجة خطورة امراض الكبد الدهنية غير الكحولية للاستغناء عن العينة الكبدية.