

Effects of Acute Pancreatitis on Plasma Total and Lipid Bound Sialic Acid Levels: An Experimental Study in Rats

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Rezumat

Efectele pancreatitei acute asupra nivelelor serice de acid sialic total și legat de compuși lipidici: un studiu experimental pe cobai

Date generale: Am investigat relația dintre nivelele serice de acid sialic total, acid sialic legat de lipide și pancreatită acută pe un model de cobai cu pancreatită acută indusă prin ligarea ductului coledoc.

Metode: Douăzeci și cinci de cobai Sprague-Dawley masculi, cu greutatea de 250-300g, au fost distribuiți în două grupuri (n=10: control, n=15: lotul de studiu). În grupul de control a fost efectuată doar laparotomie exploratorie. În grupul de studiu s-a indus pancreatită acută prin ligarea tractului comun pancreatobiliar. La 36 de ore de la intervenție cobaii au fost sacrificați, iar nivelele de amilază, acid sialic seric total, acid sialic legat de lipide și profilul lipidic au fost măsurate. Confirmarea histopatologică a pancreatitei acute a fost obținută după colorare cu hematoxilin-eozină.

Rezultate: Valorile medii ale amilazei, acidului sialic total (TSA) și acidului sialic legat de lipide (LBSA) în grupul de studiu au fost semnificativ crescute față de lotul de control. Nu au existat diferențe semnificative privind profilele lipidice din cele două grupuri.

Concluzii: Nivelele crescute de TSA și LBSA pot fi utile ca

markeri specifici în diagnosticul pancreatitei acute, independent de profilul lipidic seric.

Cuvinte cheie: Pancreatită acută, acid sialic total, acid sialic legat de lipide, profil lipidic, studiu experimental.

Abstract

Background: We investigated the relationship between serum levels of total sialic acid, lipid bound sialic acid and acute pancreatitis in a rat model of a common bile duct ligation induced acute pancreatitis.

Methods: Twenty five Sprague-Dawley male rats weighing 250-300g were divided into two groups (n=10: control, n=15: experimental). In the control group only a sham laparotomy was performed. In the experimental group, acute pancreatitis was induced by common pancreatobiliary tract ligation. After 36 hours the rats were killed and amylase, serum total sialic acid, lipid bound sialic acid and lipid profiles were measured. Histopathological confirmation of acute pancreatitis was done using hematoxylin and eosin staining.

Results: Mean amylase, total sialic acid (TSA) and lipid bound sialic acid (LBSA) measurements in the experimental group were significantly higher than in the control group. There was no significant difference in the lipid profiles between the two groups.

Conclusion: Increased levels of TSA and LBSA can be useful as specific markers in the diagnosis of acute pancreatitis independent of serum lipid profile.

Key words: Acute pancreatitis, total sialic acid, lipid bounded sialic acid, lipid profile, experimental study

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Introduction

Sialic acid is an acylated derivative of neuraminic acid with terminal carbohydrate components of glycoproteins and glycolipids. It represents the major structural component of cell membranes. Total sialic acid is bound to the lipids and proteins in the plasma and according to the attached structure also named as lipid bounded and protein bounded sialic acid. (1) Previous studies have shown that serum sialic acid levels are affected in inflammatory processes, chronic disease, high alcohol consumption and malignant disease. It is also used as an inflammatory marker in bacterial infections and chronic diseases such as pneumonia and rheumatoid processes. (2-4)

Acute pancreatitis is an inflammatory process of the pancreas. Gallstones and alcohol abuse are the most common causes of the disorder. Acinar cell injury plays a crucial role in the development of acute pancreatitis. The clinical spectrum may include edema, necrosis and hemorrhage. Various biochemical and pathological changes occur in acute pancreatitis that results from the destruction of the pancreas due to an inflammatory process. The auto-digestion of the pancreas leads to elevation of various biochemical markers. Amylase and lipase levels in serum and urine are the standard markers for diagnosis and are commonly used in acute pancreatitis (5,6). A number of serum markers have been investigated in recent years. However, sialic acid has rarely been studied.

Hence this experimental study was designed to determine the plasma levels of total and lipid bound sialic acid in pancreatobiliary duct ligation induced acute pancreatitis. Pancreatitis was demonstrated by histopathological examination. Another outcome of the study was the relationship between total - lipid bounded sialic acid and lipid profiles.

Materials and Methods

After receiving the ethical consent approval from the Animal Research Ethical Committee of Canakkale 18 Mart University Medical School, 25 male adult Sprague-Dawley rats, weighing 250–300 g, were used in the study. The animal study was conducted at the Experimental Surgery, Research and Animal Laboratory of Canakkale 18 Mart University Faculty of Medicine, Canakkale, Turkey. The experimental protocols were carried out according to the Guide for the Care and Use of Laboratory Animals (US National Institute of Health, revised 1985). All rats were housed under standard laboratory conditions with 12 h light/12 h dark cycle and allowed to have ad libitum food and water before and after surgery. During the experimental procedure, the animals were individually placed in cages and kept at room temperature (22°C). All surgical procedures were performed under sterile conditions.

Experimental protocol

Before the experimental procedure, all animals were weighed with an analytical balance and body weights were recorded. Rats were anaesthetized with intramuscular injections of

ketamine HCL (50 mg/kg, Ketalar; Parke- Davis, Morris Plains, NJ) and xylazine (10 mg/kg, Rompun; Bayer, Istanbul). Twenty five Sprague-Dawley rats were assigned in two groups, as follows:

Group 1 (control group, n=10): Only midline laparotomy was performed, and simply followed up.

Group 2 (experimental group, n=15): Acute pancreatitis was induced by ligation of the common pancreatobiliary duct, which has been shown previously to produce severe acute pancreatitis (5).

Surgical procedure

The skin of the rats was shaved and then prepared with povidone-iodine. A midline (5 cm) laparotomy was performed. After the abdominal organs were explored, the model of acute pancreatitis was induced by ligation of the common pancreatobiliary duct at the level of duodenum with 4-0 silk suture. The abdomen was closed with 3-0 silk suture. In the control group only a midline laparotomy was performed.

After 36 hours, all rats were anesthetized. Blood samples were collected via intra-cardiac route, then the rats were sacrificed. Then, the abdomen was opened. The duodenal loop with whole pancreas was harvested as a sample, for histopathological confirmation of acute pancreatitis.

Biochemical analysis

Blood samples to detect TSA and LBSA were collected in sampling tubes. Then they were immediately centrifuged at 300 g for 15 minutes. Aliquots of separated blood samples were stored at -80 C. For analysis, samples were transferred to the Cerrahpasa Medical School, Biochemistry laboratory under appropriate conditions. Levels of serum amylase and lipid profiles were determined by standard laboratory methods. CRP levels were measured immune-turbidimetrically using RANDOX analyser. For this, serum was used undiluted; CRP remains stable in the serum for 3 days at 15-25°C. The measuring range of CRP was 0-220 mg/l.

Total sialic acid and lipid-bound sialic acid assay

Total sialic acid levels were determined using the thiobarbituric acid method described by Aminoff [1] after hydrolysis of the samples in 5 unit volumes of 0.1 N H₂SO₄ at 80° for 1h. Briefly, 5 ml serum was treated with 25 ml of 25 mM periodic acid in 0.125 N H₂SO₄ (pH 1.2) for 30 min in a water-bath at 37° C. The excess of periodate was then reduced with 0.2 ml of 2% sodium arsenate in 0.5 N HCL. As soon as the yellow color of the liberated iodine disappeared (1-2 min), 2 ml of 0.1 M thiobarbituric acid was added and the test sample was covered and heated in a boiling water-bath for 7.5 min. The colored solutions were then cooled in ice-water and shaken with 5 ml of acidic butanol (1-butanol containing 5% v/v of 12 N HCL). Samples were measured at 580 nm using a spectrophotometer. A calibration curve is obtained by plotting absorbance versus concentration. As a control for measure-

ment 5, 10, 20, 30 $\mu\text{g/ml}$ solutions of N-acetyl-neuraminic acid (NANA) (Sigma Chemical Co.) were used, prepared from 40 $\mu\text{g/ml}$ stock solution. Coefficient of variation (CV %) for TSA measurement was 3.2% (mg/dl).

Lipid-bound sialic acid (LSA) was measured according to the method described by Katopodis et al., with some modifications (2,3). Forty-five μL of serum were placed in screw-capped tubes with 150 μL water. The tubes were vortexed for 10 seconds and immediately placed in ice. Three ml of cold (4°C) chloroform/methanol (2:1, v/v) mixture was added to each tube for total lipid extraction, the tubes were capped and vortexed for 30 seconds. 0.5 ml cold water was added then the tubes were recapped and mixed centrifuged for 5 minutes at 2500 rpm at room temperature. One millilitre of the upper phase was transferred to another screw-capped tube. Fifty μL of phosphotungstic acid solution (1 g/ml) was added to each tube; the tubes were vortexed and allowed to sit at room temperature for 5 minutes at 250 rpm. After that the supernatant was decanted and the remaining pellets were re-dissolved in 1 ml 37°C water by vigorously vortexing for 1 minute. For calculation of LSA values, the final experimental value was multiplied by 1.3 (a factor to correct for the volume in the extraction step). Coefficient of variation (CV %) for LSA measurement was 3.7%.

Histopathological examination

The pancreas tissue samples were placed in %10 neutral formalin solution and were routinely processed and embedded in paraffin wax. Pancreas tissue sections of 5 μm thickness were stained with hematoxylin-eosin. The specimens were examined under light microscope by the same, blinded pathologist. Acute pancreatitis was evaluated and documented in each of the tissue sections.

Statistical analysis

Data were expressed as mean + standard deviation (SD). Data were analysed using the Statistical Package for Social Sciences version 15.0 (SPSS for Windows 15.0, Inc., Chicago, IL, USA). Normality of continuous data was determined by

Kolmogorov-Smirnov test. The data were analysed Mann-Whitney test. A two tailed p value <0.05 was considered statistically significant for all comparisons.

Results

No complication was noted and no animal died during the experimental procedure. Histopathological examination of specimens revealed acute pancreatitis in the experimental group. Serum amylase levels were significantly higher in the experimental group than control group ($p < 0.001$). There was a slight increase in CRP levels however the difference was not statistically significant. Means of amylase, CRP, Total sialic - lipid bound sialic acid and lipid profile values for control and experimental groups are presented in Table 1.

Results of total sialic acid level

Serum total sialic acid levels were higher in the experimental group than in the control group. The mean levels of TSA as mg/dl in control and experimental groups were 26.02+ 8.35 mg/dl, and 161.71+31.07 mg/dl respectively. According to the results TSA levels were significantly higher in the experimental group than the control groups ($p < 0.001$, Fig. 1).

Results of lipid bounded sialic acid level

When compared with controls, the experimental group with acute pancreatitis had significantly higher values of lipid bound sialic acid levels in the serum. The means of serum LBSA levels in control and experimental groups were 11.42+1.52 mg/dl, 26.03+4.18 mg/dl respectively. There was a statistically significant difference between experimental and control groups ($p < 0.001$, Fig. 2).

Serum lipid profile

A slight decrease in HDL levels was found in the experimental group. When compared to lipid profile levels, there were no statically significant differences between control and experimental group.

Table 1. Mean and SD of total sialic acid (mg/dl), lipid bound sialic acid (mg/dl), CRP, amylase and lipid markers in sera of control and experimental groups. P value indicates level of significance

Parameters	Control (n= 10)		Pancreatitis (n= 15)		P
	Mean	SD	Mean	SD	
Total sialic acid	26.02	8.35	161.71	31.07	<0.001
Lipid-bound sialic acid	11.42	1.52	26.03	4.18	<0.001
CRP	17.10	14.04	34.20	23.74	0.009
Amylase	325.40	121.10	11215.73	3437.62	<0.001
Cholesterol	95.80	15.38	101.87	22.67	0.598
HDL	34.45	4.94	33.98	4.11	0.739
Triglyceride	73.81	4.78	72.60	10.44	0.912
LDL	68.43	12.24	66.51	12.67	0.718

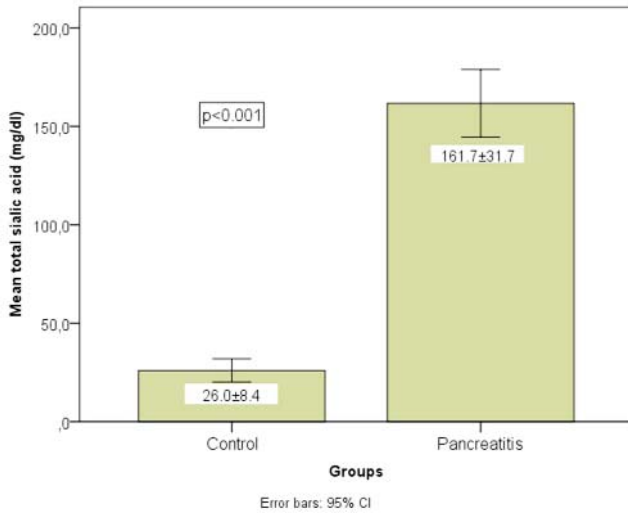


Figure 1. The serum concentrations of total sialic acid, in control and experimental groups

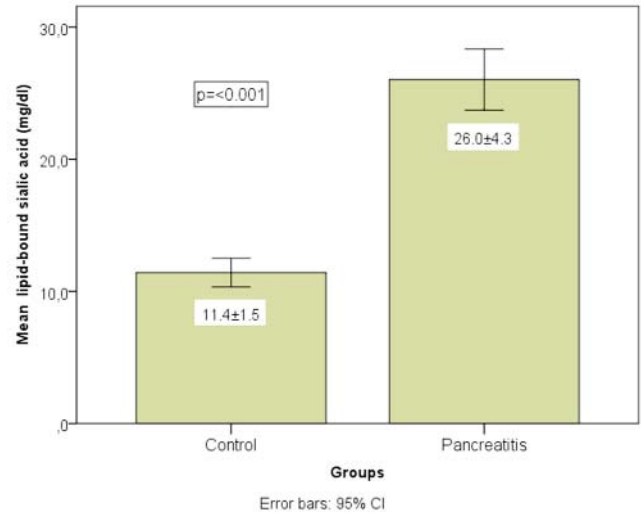


Figure 2. The serum concentration of lipid bound sialic acid in control and experimental groups

Correlation results

In this experimental study, we demonstrated that there is a positive correlation between the serum total and lipid bound sialic acid levels and amylase, CRP. We also found that serum sialic acid levels have no significant association with the lipid profile, in the early phase of acute pancreatitis. The correlations are shown in Table 2 (Figs. 3, 4 A-B).

Histopathological examination

Microscopy of the specimens of the pancreatic gland confirmed acute pancreatitis in all experimental groups, leading to interstitial edema, neutrophil infiltration, acinar necrosis and focal necrotic areas (Figs. 5, 6). The findings were determined to be consistent with pancreatitis, by a board-certified pathologist who was blinded regarding the origin of the samples.

Discussion

In this experimental study, we demonstrated that both total

sialic acid and lipid bound sialic acid levels were significantly higher in the pancreatitis group when compared to the control group. Moreover, in our study, we have clearly shown that plasma concentrations of TSA and LBSA positively correlated with the level of amylase in acute pancreatitis. The histopathological changes in the pancreas included pancreatic necrosis, and hemorrhage, and white cell infiltration.

For our experiment we ligated the bile ducts of the animals. Induction of pancreatitis via duct ligation has been shown to be an effective model in previous experimental studies reported in the literature. This model is effective as it empirically resembles the clinical portrait of gallstone associated diseases, motility disorders of the sphincter, edema and strictures at the papilla, pancreas-head tumors and similar associated conditions which are prevalently obstructive in

Table 2. The correlations between TSA-LBSA and Amylase-CRP- Lipid levels

Correlations	TSA		LSA	
	r	p	r	p
LSA	0.856	0.000		
CRP	0.202	0.332	0.471	0.017
Amylase	0.816	0.000	0.824	0.000
Cholesterol	0.097	0.643	0.185	0.376
HDL	-0.096	0.649	-0.101	0.632
Triglyceride	-0.025	0.906	0.033	0.877
LDL	-0.136	0.517	-0.006	0.979

TSA: Total sialic acid, LSA: Lipid-bound sialic acid

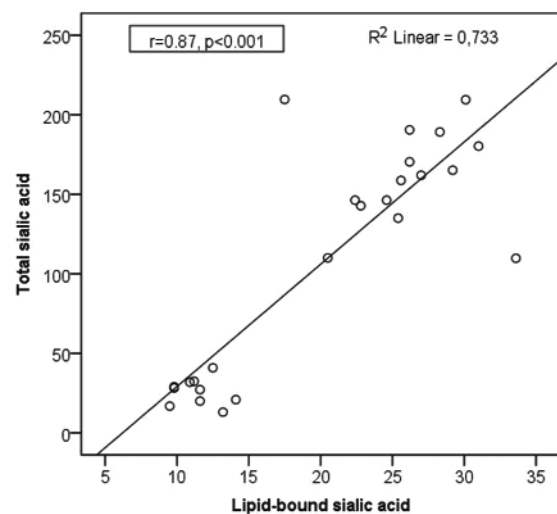


Figure 3. Positive correlation between total sialic acid and lipid bound sialic acid

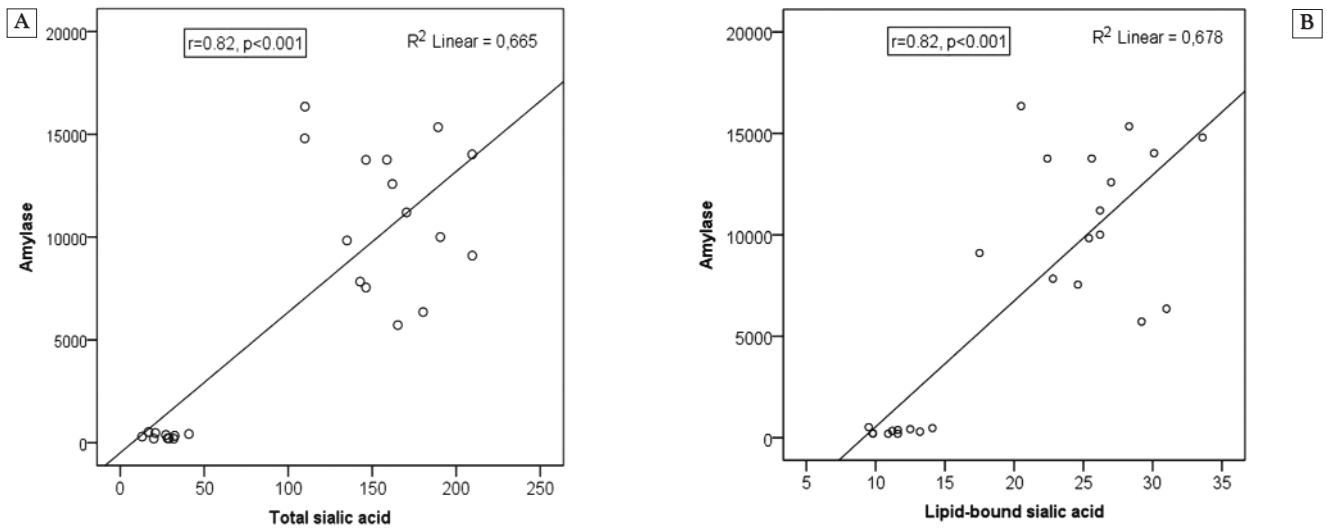


Figure 4. (A,B) Positive relationship between total-lipid bound sialic acid and amylase

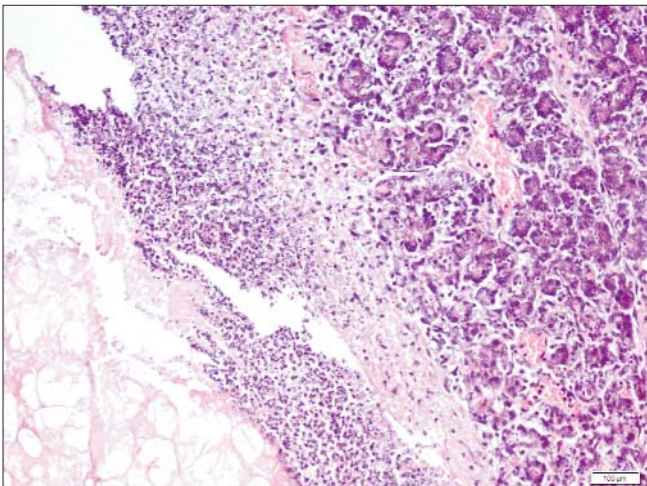


Figure 5. Hematoxylin-eosin (H-E) staining (200X). Microscopic appearance. Significant leukocyte infiltration, edema, focal fat necrosis, hemorrhage and congestion is apparent (H & E X200)

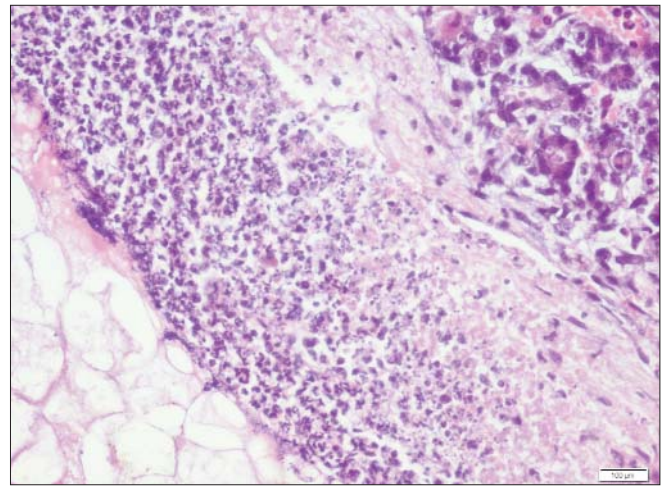


Figure 6. Hematoxylin-eosin (H-E) staining (400X). Microscopic appearance. Significant leukocyte infiltration, edema, focal fat necrosis, hemorrhage and congestion is apparent (H & E X400)

nature (5). Another reported advantage of this model is that it avoids artificial drug usage which may produce unwanted systemic effects, specifically, as would be significant in our study, the levels of a certain marker that is trying to be measured (6). Serum lipid profiles can be changed in patients with acute pancreatitis due to alcohol consumptions. For this reason, we concurred that an alcohol based pancreatitis model would be unfit, given the objectives of the study (7).

Sialic acids are structurally significant and are involved in many biological and pathological phenomena. Sialic acid residues are usually found on the external surfaces of the cells which implies a strong influence in cell biology. Desialylation has been associated with increased targeting by the immune system; a process which may be pertinent to pancreatitis pathogenesis (8).

For an understanding of the principle of how sialic acid residues and sialic acid overall is affected in inflammation, it is essential to have a basic concept of the changes which take place during inflammation, with respect to the utilization of sialic acid for functional purposes. A study conducted in Japan has demonstrated that the expression of the mRNA levels of glycosyltransferases, specifically sialyltransferases, changes during inflammation (9).

Another interesting aspect is the distribution of sialic acid residues in a polar fashion on the acinar cells of the pancreas. Using a sialic acid-specific lectin limulin (LPA, from *Limulus Polyphemus* hemolymph) the distribution of accessible sialoglycoconjugates on the surface of cells from rat and rabbit parotid gland and exocrine pancreas were analysed by Muresan et al (10). In their study, the staining

was localized only at the periphery of the acini and ducts and was absent from the apical and lateral surface of epithelial cells of the parotid gland. In the exocrine pancreas, the story was very different. The luminal surface of the cells was intensely labelled by the fluorescent lectin. This variance in distribution of sialoglycoconjugates could explain the difference in the presentation of inflammatory pathologies (10).

Thus far, sialic acids have been shown to have prognostic and informative qualities in a myriad of diseases, especially in malignancies. Increased sialic acid concentrations have been demonstrated in gynaecological cancer; (11) cancers of lung, (12) colon, (13) and ovaries; (14) urologic cancer; (15) melanoma (16). Sialic acid's utility has also been demonstrated for inflammatory processes, including deep vein thrombosis (17), myocardial infarction (18), osteoarthritis and rheumatoid arthritis (19). Few studies, however, have looked into the utility of sialic acid levels in pancreatitis.

One of the considerations that we had carefully studied was the relationship between the sialic levels and the changing lipid and cholesterol levels. Some previous studies have shown that triglyceride, free fatty acid and cholesterol levels change in acute pancreatitis (20-21,23). Furthermore, in one study published by Wakabayashi et al (22), it has been shown that sialic acid levels have a positive correlation with LDL and triglyceride levels, but a negative correlation with HDL, in disease-free patients, i.e., in patients with no severe inflammation markers; which could alter the lipid levels intrinsically. Also, sialic acid has an important role in cell functions such as immunogenicity, adhesiveness and cell recognition (22, 23). In our study, the lipid levels between the control and experimental group were not statistically significantly different. As such, the increase in lipid bound sialic acid levels cannot be attributed to variances in lipid levels.

Our study is unique due to the fact that it is the first in the literature that has systematically targeted looking into the changes in sialic acid levels in an acute pancreatitis model. There is merely one report title available in Russian literature (24) on the use of serum sialic acid level for the diagnosis of acute destructive forms of pancreatitis but there is no study so far that investigates the levels of total and lipid bound sialic acid levels and their association with acute pancreatitis.

Conclusion

Acute pancreatitis is an inflammatory condition that involves cell destruction secondary to reflux of pancreatic content. For that reason the disease can quickly progress. Increased levels of TSA and LBSA can be useful as specific markers in the diagnosis of acute pancreatitis independent of serum lipid profile.

We would like to conclude by stating that further studies are necessary to validate our findings; both by applying our findings in a clinical study with real pancreatic patients who will have altered states of metabolism (due to alcoholism), and by carrying out laboratory studies which will help elucidate the mechanism by which pancreatitis is revealed.

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Conflicts interest

Authors declare that no conflicts of interest exist.

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