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THE RELATIONSHIP BETWEEN THE LEVEL OF THIRTEEN DIFFERENT SUBSTANCES AND ENZYMES IN BLOOD AND PERITONEAL FLUID AND THE DURATION OF MECHANICAL INTESTINAL OBSTRUCTION: AN EXPERIMENTAL STUDY ON RATS

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ABSTRACT

Purpose: *The aim of this study is to analyze the relationship between the changes in blood and peritoneal fluid and the duration of mechanical intestinal obstruction.*

Methods: *A mechanical intestinal obstruction was surgically carried out in 52 Wistar albino rats. The blood and peritoneal fluid were sampled immediately (Group 1, n=13), and at 6 hours (Group 2, n=13), at 24 hours (Group 3, n=13), and at 48 hours (Group 4, n=13) following the procedure.*

Results: *Blood and peritoneal fluid ammonia concentrations, as well as blood aldolase activity have risen progressively over time ($p<0.05$), although not all pair-wise comparisons were statistically significant ($P>0.05$).*

Conclusions: *Our data suggest that peritoneal fluid and blood ammonia levels and blood aldolase levels rise according to duration of the intestinal obstruction. Since the results were not available to determine cut-off levels, future research is required to confirm these results and determine whether ammonia or aldolase can be used in clinical practice to predict the presence and duration of a mechanical intestinal obstruction.*

Key words: *Ammonia, aldolase, mechanical intestinal obstruction*

INTRODUCTION

Small bowel obstruction is an old but common problem. Intestinal obstruction is responsible for about 20% of surgical admissions for acute abdomen.[1] Most of these obstructions do not require surgical intervention; a conservative approach is generally considered to be safe. On the other hand, small bowel obstruction is associated with substantial mortality, because of the fearful complication of strangulation, which should be treated surgically.[2]

The presence of intestinal strangulation is related to the duration of the intestinal obstruction—as the duration increases, so does the risk of strangulation. However, it is not always possible for surgeons to accurately identify when the strangulation occurs.[2,3] A prospective study confirmed that the preoperative assessment of bowel strangulation by experienced surgeons was accurate in less than half of the cases[4], mainly because clinical signs and symptoms and laboratory findings do not provide sufficient evidence.[1,3]

Currently, computed tomography (CT) is used most frequently in the acute setting to answer questions relevant to the management of small bowel obstruction and to identify other causes of acute abdominal pain.[2] Although early studies indicated that CT has a high sensitivity and specificity and can accurately detect more that 90% of cases of small bowel obstruction, these reports appeared to have focused on patients with relatively high-grade obstructions, where the intestinal stream was absolutely stopped.[2,5,6] In a subsequent analysis that included patients with both low- and high-grade partial obstructions, the overall accuracy of CT was considerably lower.[7] Maglinte et al. also proposed the need for an alternative method to detect lower grades of mechanical obstruction.[7]

Many studies have been performed to evaluate the relationship between either the concentrations of some substances or the activities of some enzymes in blood or peritoneal fluid and the duration of mechanical obstruction or the presence of strangulation.[8-10] Our study examines the concentrations of a number of substances and activities of enzymes in blood and peritoneal fluid, and evaluates the relationship between their levels and the duration of the mechanical obstruction in an animal model.

MATERIAL AND METHODS

This study was approved by Education Planning Committee of Kartal Education and Research Hospital and was performed in the Experimental Medicine Research and Practice Center (DETAM) in Turkey. The study was conducted in conformity with the International Guiding Principles for Biomedical Research Involving Animals.

In this study, 52 Wistar albino rats, 10 to 13 weeks old and weighing 220 to 280 grams have been used. The animals were housed 4 to a cage, in a temperature-controlled room with 12-hour light-dark cycles, and an appropriate diet and water provided ad libitum.

A terminal ileum ligation was performed in each animal to create an experimental mechanical bowel obstruction. Intramuscular administration of a solution of ketamine (30 mg/kg) was used for anesthesia. Each animal was prepared for surgery by shaving and cleaning the lower abdomen with alcohol povidone iodine. A 30-mm long midline incision was made, and the cecum was brought into the surgical field. The terminal ileum was tied off just proximal to the ileocecal valve.

The animals were randomly divided into 4 groups consisting of 13 rats each. In Group 1, the peritoneal cavity was washed with 5 ml of saline, and intra-abdominal fluid was removed for analysis right after surgery. Blood was also obtained at that time by directly cannulating the heart.

In the remaining 39 rats, the fascia and skin were closed with 3/0 chromic catgut and 3/0 silk. A re-laparotomy was performed 6 hours (Group 2, n=13), 24 hours (Group 3, n=13), and 48 hours (Group 4, n=13) after the initial operation. The peritoneal fluid and blood samples were collected in the same manner as Group 1. After re-laparotomy, the rats were sacrificed with an overdose of phenobarbital as well.

All samples were transferred to a laboratory in a refrigerator that was maintained at a temperature of 4°C. The parameters that were analyzed in blood and peritoneal fluid were as follows: the concentration of substances (ammonia, bilirubin, creatinine, glucose, urea) and the activities of enzymes [alanin aminotransferase (ALT), aldolase, alkaline phosphatase, amylase, aspartate aminotransferase (AST), creatinine phospokinase (CPK), gamma-glutamic transferase (GGT), lactic dehydrogenase (LDH)]. The analyses were done either manually or automatically in a Roche / Hitachi system machine (Roche Diagnostic, Mannheim, Deustchland).

Statistical analysis: The statistical analysis was performed with SPSS (Statistical Package for Social Sciences) for Windows version 10.0. The data are presented as median and range, if the distribution is wide (if standard deviation is higher than one third of the mean value); otherwise presented as mean and standard deviation. The overall comparison was confirmed with the nonparametric Kruskal-Wallis test, and Mann Whitney U Test was used to show the difference between the groups. A P value of 0.05 or less was considered statistically significant.

Table 1. The Blood Concentrations of the Substances and the Activities of the Enzymes

	Group 1	Group 2	Group3	Group 4	p
Substances					
Ammonia	92; 0-253	110; 36-241	181; 101-343	240; 94-365	P<0.001
Bilirubin	0.05; 0-1.6	0.07; 0-1.1	0.07; 0-1.1	0.06; 0-1.0	P=0.782
Creatinine	0.31; 0.11-0.87	0.35; 0.08-1.21	0.86; 0.05-1.56	0.65; 0.10-1.15	P=0.062
Glucose	120.7±29.0	139.6±18.8	151.2±41.5	114.7±38.7	P=0.056
Urea	38.7±10.7	42.0±5.1	46.8±14.1	42.5±11.4	P=0.465
Enzymes					
ALT	65.4±24.7	59.5±12.0	71.4±29.5	70.1±10.1	P=0.11
Aldolase	42; 25-64	70; 24-109	104; 66-139	168; 120-188	P=0.017
Alkaline Phosphatase	192; 30-337	190; 27-290	198; 17-298	180; 43-280	P=0.973
Amylase	1545; 130-3350	1733; 470-2380	1715; 800-3100	1764; 250-2980	P=0.841
AST	263.3±44.1	262.4±38.5	265.1±91.2	281.8±43.7	P=0.10
CPK	225; 5-350	196; 35-350	249; 30-400	290; 0-570	P=0.230
GGT	0.25; 0-1.3	0.14; 0-0.9	0.14; 0-1.1	0.63; 0.2-1.3	P=0.072
LDH	2330.5±468.2	2324.6±242.5	2440.9±380.5	2202.6±502.7	P=0.912

(ALT: Alanin aminotransferase, AST: Aspartate aminotransferase, CPK: Creatinine Phosphokinase, GGT: Gamma-glutamile transferase, LDH: lactic dehydrogenase) The data are presented as median and range, if the distribution is wide (if standard deviation is bigger than one third of the mean value); otherwise presented as mean and standard deviation. The comparison was confirmed with the nonparametric Kruskal-Wallis test and a P value of 0.05 or less was considered statistically significant.

RESULTS

No animal died during the study.

Table 1 summarizes the blood results. Blood ammonia concentrations and aldolase activities appeared to have increased, in relation to the duration of the obstruction (p<0.01). The blood ammonia concentrations of all animals were given in Figure 1. The increase was not significant during the first 6 hours, but the pair-wise comparison between groups 2, 3 and 4 were highly significant (p<0.05 for all). The blood aldolase activities were given in Figure 2. As ammonia concentrations, blood aldolase activities were increasing over time (p<0.05 for all), there was no statistical difference between group 1 and 2.

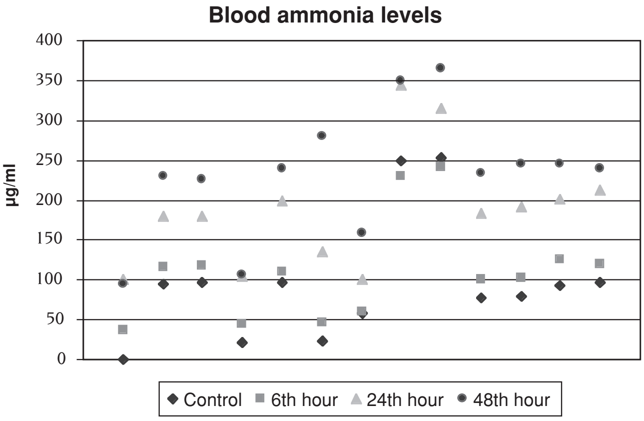

Figure 1. Blood Ammonia Concentrations in Groups

[Pair-wise comparison (analyzed with Mann Whitney U test): Group1 vs 2, P=0.158, Group 1 vs 3, P=0.001, Group 1 vs 4, P=0.002, Group 2 vs 3, P=0.024, Group 2 vs 4, P=0.020, Group 3 vs 4, P=0.061)]. A p value less than 0.05 is significant.

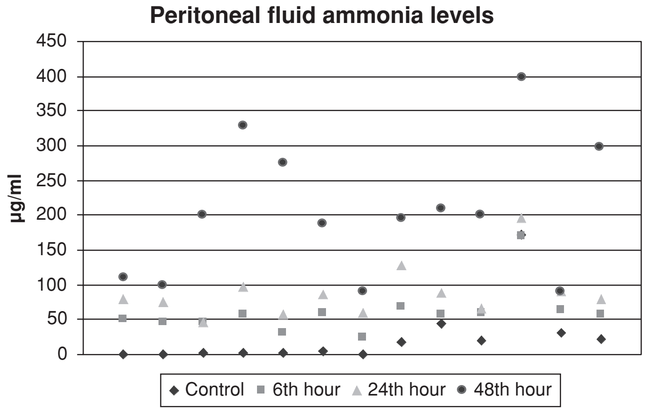

Figure 2. Peritoneal Fluid Ammonia Concentrations in Groups

[Pair-wise comparison (analyzed with Mann Whitney U test): Group1 vs 2, P=0.001, Group 1 vs 3, P=0.001, Group 1 vs 4, P=0.001, Group 2 vs 3, P=0.006, Group 2 vs 4, P=0.001, Group 3 vs 4, P=0.001]. A p value less than 0.05 is significant.

The concentration of all substances and activities of all enzymes in peritoneal fluid were given in Table 2. Peritoneal fluid ammonia levels increased, in close relation to the duration of mechanical obstruction (p<0.01 for overall comparison and for all pair-wise comparisons) (Figure 3).

Table 2. Peritoneal Fluid Concentrations of the Substances and the Activities of the Enzymes

	Group 1	Group 2	Group3	Group 4	p
Substances					
Ammonia	4; 0-173	58; 24-169	80; 47-197	200; 90-399	P<0.001
Bilirubin	0.0; 0-0.02	0.0; 0-0.03	0.0; 0-0.23	0.0;0-0.15	P=0.800
Creatinine	0.01; 0-0.10	0.00; 0-0.09	0.00; 0-0.05	0.00; 0.001	P=0.670
Glucose	0; 0-35	0; 0-30	0; 0-78	0; 0-56	P=0.856
Urea	2.5; 0-19.8	2.4; 0-18.0	4.5; 0-16.3	2.8; 0-34.6	P=0.110
Enzymes					
ALT	0; 0-2	0; 0-0	0; 0-17	0; 0-10	P=0.689
Aldolase	24; 0-57	14; 0-52	20; 0-43	1; 0-68	P=0.207
Alkaline Phosphatase	6.1; 0-70.4	22.3; 0-67.9	19.7; 0-56.8	13.7; 0-112.2	P=0.180
Amylase	0; 0-178	45; 0-230	120; 0-450	105; 0-400	P=0.056
AST	0; 0-10	0; 0-0	0; 0-25	0; 0-34	P=0.950
CPK	0; 0-120.3	0; 0-130.5	0; 0-245.0	0; 0-20.0	P=0.780
GGT	0; 0-0.01	0; 0-0.01	0; 0-0.08	0; 0-0.01	P=0.620
LDH	0; 0-1100	0; 0-1700	30; 0-2300	32; 0-1230	P=0.564

The data are presented as median and range, if the distribution is wide (if standard deviation is bigger than one third of the mean value); otherwise presented as mean and standard deviation. The comparison was confirmed with the nonparametric Kruskal-Wallis test and a P value of 0.05 or less was considered statistically significant.

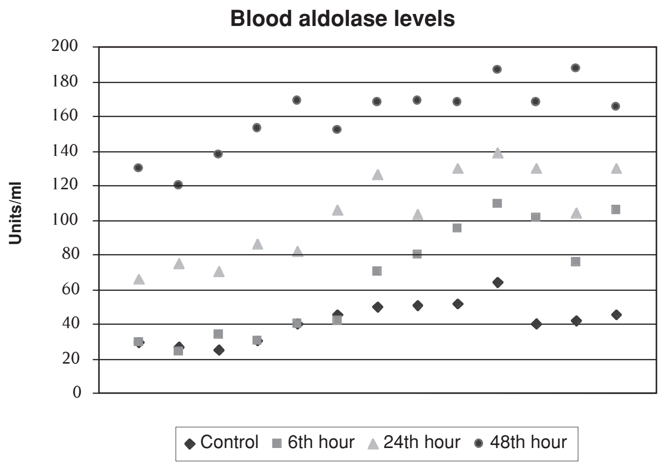

Figure 3. Blood Aldolase Activities in Groups

[Pair-wise comparison (analyzed with Mann Whitney U test): Group1 vs 2, P=0.150, Group 1 vs 3, P=0.001, Group 1 vs 4, P=0.001, Group 2 vs 3, P=0.008, Group 2 vs 4, P=0.001, Group 3 vs 4, P=0.001]. A p value less than 0.05 is significant.

DISCUSSION

It is difficult for clinicians to make an early and accurate diagnosis of complete mechanical intestinal obstruction in patients who have been admitted with signs and symptoms of intestinal obstruction. Unfortunately, we have no simple and reliable method for detecting mechanical obstructions that will not respond to conservative management. Many studies have already addressed the value of preoperative laboratory values in predicting mechanical obstructions.[4,8,11-14] But none of them has been shown to be accurate enough for clinical practice.

We have studied 13 different variables (5 different substances and 8 enzymes) in blood and peritoneal fluid. Two of these substances—ammonia and aldolase—have shown considerable promise as an index for the duration of intestinal obstruction.[8,10] In the study by Mansberger[10], increased ammonia levels in the peritoneal fluid of patients with strangulated or perforated bowels were found. However, that study was limited by the fact that ammonia levels in the peritoneal fluid were collected only once and not repeated. Also, simultaneous blood ammonia levels were not measured.[10] However, the progressive increase of the levels of the substances should be emphasized to determine the relationship between the levels and the duration of the mechanical obstruction. By better understanding this relationship, we believe that these two substances could possibly be utilized to predict the duration of mechanical intestinal obstruction—and therefore the need for surgical or conservative treatment.

Many sources such as the liver, kidney, muscle, brain, heart, blood, and intestinal microflora produce ammonia.[10,15] Ammonia concentrations in the peritoneal fluid increase with any condition that results in the transudation or spillage of intraluminal intestinal contents into the peritoneal cavity.[10] In our study, the increase of ammonia levels in the peritoneal fluid was progressive and prominent in every step of the evaluation. The elevation was statistically significant within hours after the onset of the obstruction (P<0.01 for the comparison of Group 1 versus Group 2) and continued to the late phases of mechanical obstruction (P<0.01 for Group 2 versus 3 and Group 3 versus 4).

Simple intestinal obstruction of the small bowel is usually associated with bacterial translocation, which may have contributed to the increase in ammonia levels in our study.[16] Blood ammonia levels seemed to rise in conjunction with peritoneal fluid ammonia levels. But the change in blood that occurred between Group 1 and 2 was not statistically significant. This may be related to the fact that blood ammonia concentrations do not immediately reflect changes in peritoneal fluid ammonia concentrations. The increase in blood ammonia concentrations was not persisting statistically significant on a long-term basis (P>0.05 for Group 3 versus 4).

Aldolase plays an important role in glucose metabolism. It is present in all body cells and occurs in particularly large quantities in the muscles, liver, and brain. Serum aldolase activity increases after myocardial infarction or with myotonic muscular diseases such as progressive muscular dystrophy and polymyositis. The enzyme rises to very high levels in acute hepatitis, and slightly elevates in patients chronic hepatitis and obstructive jaundice.[17] In our study, aldolase levels in the peritoneal fluid decreased over time, probably because the small bowel, which is the most affected organ in a mechanical obstruction process, was not a source of aldolase. Blood aldolase levels significantly increased over time except in the early phase (P>0.05 for Group 1 versus Group 2; P<0.01 for other pair-wise comparisons). This might be secondary to the toxic effect of intestinal obstruction on liver, because we also observed mild but not significant increases in ALT, AST and GGT in our study.

We evaluated 13 different substances and enzymes in our study. Most of them were not correlated with the duration of mechanical obstruction. Blood creatinine and glucose concentrations were likely to elevate over time, but it was not significant. The increases in these substances might be related to the dehydration of the animals, which is very common during the mechanical intestinal obstruction. Although they were not significant, the activities of AST, ALT and GGT in blood also increased especially during the late phase of intestinal obstruction (beginning with 24 hours after the operations), which might be related to the possible toxic effect of intestinal obstruction on the liver. In addition, we also observed a progressive increase of amylase concentrations in peritoneal fluid, but it was not significant, either.

The most important lack of our data was the wide distributions in the results of the groups. This deficit was present even when the results were highly significant. For that reason it was impossible to find out any "cut-off" levels in the measurement of any variables. For example: although the ammonia concentrations in blood were significantly different in pair-wise comparisons and between the levels in 6^{th} and 48^{th} hours, almost all animals in group 2 (levels at 6^{th} hour) had higher concentration levels than half of the animals in group 4 (levels at 48^{th} hour) (Table 1 and Figure 1). This is an important issue that limits the use of these substances and enzymes in clinical practice especially in the patients who are admitted to emergency services with suspected mechanical obstructions.

In our opinion, these preliminary results suggest that blood and peritoneal fluid ammonia concentrations and blood aldolase activities rise with the duration of an intestinal obstruction in a rat model. Since the distributions of the results in the groups are quite wide, future research is needed to confirm these results and expand on our hypothesis that ammonia and aldolase might be used to predict the duration of an intestinal mechanical obstruction in human beings.

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