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Serum tumor necrosisfactor-α, glutamate and lactate changes in two different stages of mechanical intestinal obstruction

Mekanik intestinal obstrüksiyonun iki farklı evresinde serum tümör nekroz faktör alfa, glutamat ve laktat değişiklikleri

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Background/aims: *Mechanical intestinal obstruction is a difficult-to-diagnose surgical emergency, especially in the early stage. Clinical and radiological evaluation are the main methods for the diagnosis. Metabolic, inflammatory and ischemic changes occur during intestinal obstruction. No specific biochemical parameters related to diagnosis and severity of intestinal obstruction have been found. In this experimental study, we investigated serum tumor necrosis factor a L-glutamate and L-lactate levels as biochemical markers which reflect physiopathological processes in two different stages of intestinal obstruction. Methods: Mechanical obstruction was created in Wistar rats with distal Heal ligation. Animals were divided into five groups (Control, Sham-12, Sham-24, Intestinal obstruction 12 and intestinal obstruction-24) and each group consisted of 10 rats. Blood samples were taken 12 and 24 hours after Sham and intestinal obstruction operations. Tumor necrosis factor a levels were measured by ELISA. Serum L-glutamate and L-lactate levels were measured by colorimetric method.* ***Results:*** *Glutamate levels were significantly high especially in the early stage, whereas tumor necrosis factor a increase was significant only in late stage of intestinal obstruction. Serum lactate levels were similar among the groups.* ***Conclusion:*** *Serum glutamate levels may have a potential role as a biochemical parameter contributing to diagnosis, especially in the early stages of intestinal obstruction.*

Key words: Intestinal obstruction, TNF, glutamate, lactate

Amaç: *Mekanik intestinal obstrüksiyon, özellikle erken evrede tanısı güç olan cerrahi bir acildir. Tanı, esas olarak klinik ve radyolojik verilerle konulur. Fizyopatolojik süreçte metabolik, inflamatuvar ve iskemik değişiklikler gözlenir. Henüz hastalığı ve şiddetini gösteren özel bir biyokimyasal parametre bulunmamaktadır. Bu deneysel çalışmada, bu süreçleri yansıtan 3 bağımsız biyokimyasal parametre (tümör nekroz faktör-a L-glutamat ve L-laktat), hastalığın 2 farklı döneminde araştırılmıştır.* ***Yöntem:*** *Her biri 10 Wistar rattan oluşan 5 grup oluşturuldu (Kontrol, Shaml2, Sham24, Intestinal obstriksiyon-12, Intestinal obstriksiyon-24). Sham gruplarında laparatomiden 12 ve 24 saat sonra; mekanik intestinal obstriksiyon gruplarında (intestinal obstriksiyon-12, Intestinal obstriksiyon-24) distal ileal ligasyondan 12 ve 24 saat sonra kan örnekleri alındı, tümör nekroz faktör alfa düzeyleri ELISA ile; L-glutamat ve L-laktat düzeyleri kolorimetrik yöntemle ölçüldü.* ***Bulgular:*** *Intestinal obstriksiyon'nun erken döneminde L-glutamat; geç döneminde tümör nekroz faktör alfa düzeyleri anlamlı yüksek bulundu. L-laktat düzeyleri bakımından gruplar arasında fark yoktu. Sonuç: Serum glutamat değerleri, intestinal obstriksiyon'nun özellikle erken dönemlerinde tanıya katkı sağlayıcı biyokimyasal bir parametre rol oynayabilir.*

Anahtar sözcükler: Intestinal obstrüksiyon, TNF, glutamat, laktat

INTRODUCTION

Mechanical bowel obstruction (MBO) is one ofthe most important emergency surgical indications. Although the edidogicel spectrum is wide, the physiological course follows similar pathways. The diagnosis is usually based on clinical and radiological evaluations, yet no specific biochemi cal parameter exists regarding early diagnosis of MBO.

In ongoing MBO, intestinal absorptive and secre tive functions deteriorate which results in pro gressively impaired fluid and electrolyte balance. Nevertheless, ifthe obstruction is not relieved in a timely manner, mucosal ischemic injury (due to the increased intraluminal pressure and consecu tive bacterial translocation) and sepsis are

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inevitable (l, 2). Significant bowel wall ischemia results in perforation and secondary peritonitis occurring in the earlier periods of the disease, particularly in the cases of MBO developing strangulation (3, 4). Thus, metabolic imbalance combined with ischemic wall injury carries critical mortality and morbidity risk which clearly points to the importance of early diagnosis and treatment (5).

In this experimental study, three independent biochemical parameters, L-glutamate, L-lactate and tumor necrosis factor-a (TNF-a), predicting intestinal metabolism, ischemia and inflammation, respectively, were evaluated at two different time points after MBO (after 12 and 24 hrs). The aim of the study was to investigate the role of the above-mentioned biochemical parameters in the early diagnosis of MBO.

MATERIALS AND METHODS

Adult female Wistar rats weighing 180-210 gr were maintained on diets ad libitum in diurnal lighting conditions. All animals were housed in a standard animal room and operated under ether anesthesia. The local ethical committee approved the study.

A) Experimental Design:

The rats were divided into five groups (each included 10 rats). Blood samples were collected by means of cardiac puncture and animals were sacrificed at the end of the study. Blood samples were centrifuged and stored at -70°C until the biochemical analysis.

Study Groups:

Control Group: In this group, only blood samples were drawn from the rats.

Sham-12 Group: Laparotomy was performed in this group. Normal saline was injected subcutaneously at a dose of 3 ml/kg after the abdomen was closed without any surgical manipulation. Blood samples were collected at the 12 hour after the surgical procedure.

Sham-24 Group: The same procedures in the Sham-12 group were applied in this group, but blood samples were collected at the 24th hour after surgery.

Intestinal Obstruction 12 (IO-12) Group: In this group, 1 cm proximal to the ileo-cecal valve was sutured with 3/0 silk to develop simple mechanical intestinal obstruction. Normal saline was injected subcutaneously at a dose of 3 ml/kg just after the abdomen was closed. Blood samples were taken at the 12th hour after the laparotomy.

Intestinal Obstruction 24 (IO-24) Group: In this group, the same procedures in the IO-12 group were applied, but blood samples were taken at the 24th hour after the laparotomy.

B) Biochemical Analysis:

Analysis of serum TNF-a: Standard Biomar TNF-a measuring kit (ER-TNFA) was used to measure plasma TNF-a levels. This test is based on "sandwich enzyme immunoassay". The results were compared with standardized curve at 450 nm.

Serum values of L-glutamate were measured by colorimetry using

Boehringer-Mannheim L-glutamic acid kit (Cat. No: 139092).

Serum values of lactic acid were measured by colorimetry using

Boehringer-Mannheim L-lactic acid kit (Cat. No: 139092).

C) Statistical Analysis:

Analysis of variance (ANOVA) test was used to compare the groups. Tukey test was used to compare the groups which were found to be significant. For all of the statistical analysis, p values less than 0.05 ($p<0.05$) were accepted as significant.

RESULTS

TNF-a **values:** Average plasma levels of TNF-a were as follows (results are shown as average ± standard error): 77.5 ± 23.5 pg/ml in the control group, 112.7 ± 59.5 pg/ml in the Sham-12 group, 153.1 ± 44 pg/ml in the Sham-24 group, 285.2 ± 170.2 pg/ml in the 10-12 group and 269 ± 60.1 pg/ml in the IO-24 group. No significant difference was found when the groups in which the blood samples were collected at the 12th hour after the surgery (Sham-12 and IO-12 groups) were compared with the control group ($p=0.096$). It was found that serum levels of TNF-a in the groups in which the blood samples were collected at the 24th hour after surgery were significantly higher than in the control group ($p=0.016$). Furthermore, the serum values of the IO-24 group were found to be

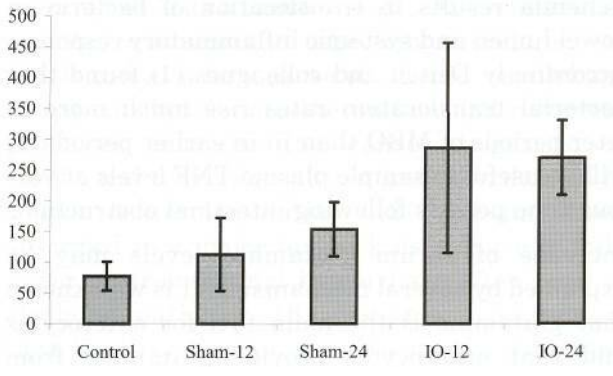

Figure 1. TNF: Tumor necrosis factor levels. IO: intestinal obstruction.

significantly higher than the values in the Sham-12 group (p= 0.037). Serum TNF-a levels of each group are shown in (Figure 1).

L-glutamate values: Average serum levels of L-glutamate were as follows: 0.056 ± 0.0057 mmol/L in the control group, 0.042 ± 0.014 mmol/L in the Sham-12 group, 0.092 ± 0.015 mmol/L in the Sham-24 group, 0.130 ± 0.015 mmol/L in the IO-12 group and 0.09 ± 0.007 mmol/L in the IO-24 group. When serum levels of L-glutamate of the groups in which the blood samples were drawn at the 12th hour after surgery were compared, it was found that the values of the IO-12 group were two fold higher than the other two groups (p=0.003). When serum levels of L-glutamate of the groups in which the blood samples were drawn at the 24th hour after surgery were compared, it was found that the levels of the IO-24 group were different from the control group (p=0.022), but not from the levels of the Sham-24 group (p=0.061). Serum L-glutamate levels of each group are shown in Figure 2.

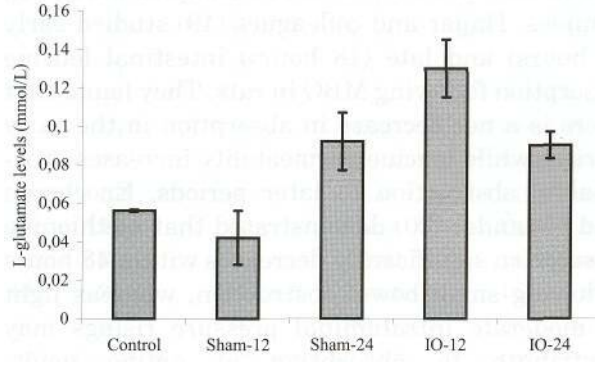

Figure 2. L-glutamete levels. IO: intestinal obstruction.

L-lactate values: Serum levels of L-lactate were as follows: 31.06 ± 3.14 mmol/L in the control group, 34.2 ± 3.57 mmol/L in the Sham-12 group, 31.8 ± 5.6 mmol/L in the Sham-24 group, 35.08 ± l mmol/L in the IO-12 group and 35.04 ± 2.95 mmol/L in the IO-24 group. No significance was found among the groups (p=0.87). Results are shown in (Figure 3).

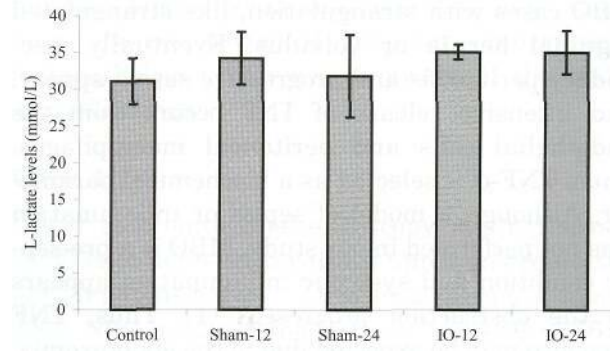

Figure 3. L-lactate levels. IO: intestinal obstruction.

DISCUSSION

The main purpose of our study was not to test the diagnostic success of the selected biochemical parameters on two different periods of MBO. Certainly, clinical and radiological evaluations are of great importance in diagnosis of such cases. But, the biochemical parameters we tested may have contributions in this process. Two Sham groups, different from the control, were added to the study. The aim of this was to compare the measurements in which bowel obstruction developed with the measurements in the cases on which only surgical intervention (abdominal opening and closure) was applied. Of course, an idealized biochemical parameter is different from the control as well as surgical trauma. In the clinical observations, mortality increases parallel to the duration of the mechanical obstruction of the bowel (5). Thus, the biochemical parameters tested in our study were evaluated at different periods of the obstruction process.

Infection and inflammation develop by two pathophysiological processes in MBO. The first of these is bacterial translocation due to MBO. In this process, the pathogenic microorganisms in the bowel lumen pass into the portal and systemic circulation through the normal intestinal mucosa in such situations like MBO, trauma, total parenteral nutrition, burns and biliary narrowing. Particularly, gram-negative bacteria and their

endotoxins translocate (1, 6-8). These, in turn, induce release of the pro-inflammatory cytokines, especially TNF-a and interleukin-1 (IL-1). Of these, TNF-a in particular is of great importance to begin the cascade of sepsis and multiple organ failure in the pathologies in which bacterial translocation develops (2, 9). Secondly, ischemia of the bowel wall becomes more important, and perforation of the bowel wall develops rapidly in the MBO cases with strangulation, like strangulated inguinal hernia or volvulus. Eventually, secondary peritonitis and progressive sepsis appear, and intensive release of TNF occurs from the endothelial cells and peritoneal macrophages. Thus, TNF-a is selected as a biochemical parameter. Although a model of sepsis or inflammation was not performed in our study, MBO is a pre-septic condition and systemic inflammation appears as the obstruction progresses (1). Thus, TNF response may be expected due to the endotoxemia.

There are numerous clinical and experimental studies regarding the release of cytokines after MBO. For instance, Morris et al. (10) studied the serum TNF levels of a number of horses with gastrointestinal pathologies. Higher TNF levels were found in the horses with MBO and even higher levels were seen in those horses with inflammatory bowel disease or intestinal obstruction with strangulation. Willetts and colleagues (11) found higher plasma cytokine concentrations in children with ileo-colic invagination.

We have found that serum concentrations of TNF increase, particularly in the late periods of MBO, while serum levels of glutamate increase earlier in MBO. It was found that average TNF values at the 12^{th} hour after intestinal obstruction are higher than Sham samples at the 12^{th} hour. But the difference between them was not statistically significant. This seems to be due to a high standard of error of TNF means in the IO-12 group. In other words, there are significant differences among TNF values in the IO-12 group. Of course, increasing the size of the samples or creating the groups again may be useful. Although speculative, the time period in which the sampling procedures were performed may be the period in which the values of TNF began to rise. In the view of the fact that TNF is the main pro-inflammatory cytokine and endotoxin is the main factor inducing release of it, the main bacterial inflammatory response may be expected to develop following mucosal ischemia in later periods of MBO. Furthermore, impaired mucosal continuity following mucosal ischemia results in translocation of bacteria in bowel lumen and systemic inflammatory response. Accordingly Deitch and colleagues (1) found that bacterial translocation rates rise much more in later periods of MBO than in in earlier periods. It will be useful to sample plasma TNF levels at various time periods following intestinal obstruction.

Increase of serum glutamate levels may be explained by several mechanisms. It is well known that glutamine is the main fuel for enterocytes and that enterocytes provide glutamine from bowel lumen under normal conditions. Presence of glutamine is essential to maintain intestinal absorption as well as to provide enteric crypt cellular proliferation and mucosal integration (12). Adverse events, such as distention following intestinal obstruction, fluid retention and mucosal impairment, may be corrected by a diet containing glutamine. Apart from these, immune cells such as neutrophiles, monocytes and macrophages are dependent on glutamine for their development and activations (14, 15). The organism must thus provide the glutamine to maintain intestinal homeostasis and to meet the fuel requirements of the cells in earlier periods. Glutamine is liberated to plasma in large amounts from various sources, of which the most important is skeletal muscles, following trauma or catabolic processes. In such processes glutamine consumption in the bowel rises (16). Liberated glutamine joins several processes in the liver. For instance, through glyconeogenesis, urogenesis, glutathione synthesis and tricarboxylic acid cycle (17), glutamine is converted to glutamate and NH3 by the enzyme glutaminase (18).

On the other hand, it is likely that there may have been an increase of glutamine absorption from the bowel segments other than the obstruction site in the 12-hour period in which we sampled the serum samples. Hajjar and colleagues (19) studied early (2 hours) and late (18 hours) intestinal leucine absorption following MBO in rats. They found that there is a net decrease in absorption in the early period, while leucine permeability increases proximal to obstruction in later periods. Enochsson and Nylander (20) demonstrated that methionine absorption significantly decreases within 48 hours following small bowel obstruction, whereas light to moderate intraluminal pressure risings may contribute to absorption of amino acids. Additionally, manipulations of bowels may be

regarded as a factor influencing the mucosal permeability. There are numerous studies proving this. For instance, Schwarz and colleagues (21) demonstrated that intestinal manipulations may significantly increase mucosal permeability, and that these manipulations exacerbate paralytic ileus following surgery. Thus, when one considers that intestinal manipulation did not occur in our Sham groups, it can be concluded that this situation may be an important factor in increasing serum glutamate levels in IO intestinal obstruction.

It has been proposed that serum lactic acid levels may be an accurate biochemical parameter in advanced ischemic events. Lange and Jackel (22), in their prospective studies carried out in two different clinics, found that plasma lactate concentrations are the "best marker" reflecting the degree of ischemia in patients with mesenteric ischemia. The model created in the current study is not a strangulation model. But, ischemic changes are seen in pathologies in which IO develops. Intraluminal pressure rises and mucosal ischemia develops proximal to mechanical obstruction. It was found that regional blood flow of the bowel significantly decreases when the intraluminal pressure reaches 40 mmHg (23). In the present study, no significant difference could be found between groups regarding plasma lactate concentrations. It may be due to the fact that mucosal ischemia develops much later than IO. For instance, intraluminal pressure following IO reaches 40 mmHg within three days (24). But in the present study, serum samplings were performed at the 12^{th} and 24^{th} hours after the surgery. Another contributory factor is that a liver showing normal performance can metabolize serum lactate until it reaches a threshold level. In other words, the liver may mask high lactate levels (25). In our groups, the serum lactate levels following IO likely remained within the metabolic limits mentioned above. Higher levels may be expected in IO cases with strangulation, in advanced periods of obstruction or when general ischemia is more prominent. This requires further study. In any case, it seems reasonable to conclude that lactate is a suitable biochemical parameter for predicting intestinal obstruction or in the follow-up of IO.

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