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Serum D(-)-Lactate and Nitric Oxide (NO) Levels in Acute Intestinal Ischemia

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Abstract: To determine serum D(-)-lactate and NO levels in patients with acute mesenteric ischemia. 22 patients (12 with acute mesenteric ischemia, 10 with bowel obstruction) and 12 control patients who were operated due to goitre. Serum D(-)-lactate and NO levels in acute mesenteric ischemia cases compared to small bowel obstruction cases and control patients. Increased serum D(-)-lactate and NO levels in serum levels of ischemic bowel were found to be significantly increased compared to both the cases with small bowel obstruction and control subjects (for D(-)-lactate: $p<0.01$, $p<0.0001$; for NO $p<0.01$, $p<0.0001$, respectively). Significant correlations between serum D(-)-lactate and NO levels in both acute mesenteric ischemia and small bowel obstruction group ($r=0.506$, $p<0.05$, and $r=0.692$, $p<0.05$, respectively). The sensitivity and the specificity of the D(-)-lactate assay were 75% and 90%, respectively. The sensitivity and the specificity of NO assay were 83% and 80% respectively. The positive predictive values of the D(-)-lactate and NO were 81% and 71%, respectively. Determination of serum D(-)-lactate and NO levels may be helpful in the diagnosis of difficult acute mesenteric ischemia cases.

Introduction

Since only very few diagnostic tests are specific for mesenteric infarction in its early stage, this disease has a high mortality rate that has been reported as 85% in the literature (1). The diagnosis of acute intestinal ischemia is very difficult due to not only nonspecificity of sign and symptoms of the disease but also the presence of additional medical problems seen in most of the patients(2). For solving this problem, many investigators have attempted to find a simple laboratory test that could identify ischemic bowel before irreversible damage occurs. In this respect, Jamieson and colleagues reported that serum phosphate levels were found to be increased in dogs with severe intestinal necrosis (3) and some investigators focused on intracellular enzymes released by the ischemic bowel (4). Unfortunately, up to now, no marker has been found to be useful in clinical studies (5). Knowledge about the production of D-lactic acid by bacteria, its absorption from the intestinal tract, and its concentrations in various body fluids may help to identify the significance of findings of this metabolite in an ill patient (6).

NO is generated from L-arginine by the action of the enzyme NO synthase (NOS) in vascular and neural tissues of many organs (7,8). It has a role in vasodilation, platelet aggregation and acts as an effector molecule in mediating cytotoxicity (8,9). NO is a lipophylic gase (8) and its synthesis is stimulated by endotoxins and ischemic conditions (10,11). Although several studies have shown NO to be protective by acting as a vasodilator to prevent long-term ischemia, some studies have suggested that NO could contribute to ischemia/reperfusion (I/R) injury (12). While NO, since it is physiologically vasodilatator, has a role in protecting tissue in ischemic events, and since it is chemically free radical, NO may occasionally be harmful to tissue by producing secondary toxic products. In low tissue O2 saturation condition, NO reduces ischemic damage by increasing blood flow secondary to vasodilating effect. In high tissue O_2 saturation condition, it leads to directly toxic metabolites to a cell by reacting superoxide and forming some toxic substances such as peroxynitrite (13).

D(-)-lactate is the stereoisomer of the mammalian L(+)-lactate. D(-)-lactate is not produced by mammalian tissue, but it may be unmetabolized or only slowly metabolized by human tissues (14). In this study, we

aimed to investigate whether the determination of serum D(-)-lactate and NO levels are useful markers for early diagnosis of acute intestinal ischemia.

Materials and Methods

This study was carried out in the Department of General Surgery, School of Medicine, Atatürk University. A preoperative blood sample was obtained from 22 patients undergoing surgery for acute abdominal emergencies including suspected acute mesenteric ischemia. Blood samples were not analyzed for patients who had abdominal pain and did not warrant a laparatomy or for patients undergoing elective laparatomy. As a control, blood samples were obtained from 12 patients having non-abdominal elective surgery for nodular goitre.

Serum D(-)-lactate levels were studied according to Brandt et al's method. Firstly, serum samples were deproteinized by perchloric acid and potassium hydroxide. Then, serum D(-)-lactate level was detected using a previously described spectrophotometric metod (15). In this method, briefly, the absorbance of NADH formed from NAD in the presence of L-lactate andLDH is read at 340 nm (Shimadzu Spectrophotometer UV-120-01). The results are presented as g/L.

For serum levels of nitric oxide determination, the serum samples obtained were deproteinized with sulfosalicylic acid (35%). Then, nitrate (NO^-_3) in a supernatant was reduced by cadmium column (100 mesh) to nitrite (NO^-_2) and the concentration of NO^-_2 was determined with the Griess reaction (16). In this reaction, the Griess reagent consisted of one part 0.1% naphthylenediamine dihydrochloride and one part 1 % sulfanilamide in 5% phosphoric acid, mixed together and kept chilled. After incubation in a waterbath at 60°C, the color of the product dye was developed and its absorbance was detected at 560 nm by spectrophotometer. Standards of sodium nitrite and sodium nitrate ranged from 10 to 60 nmol/mL. NO values was given as NO^-_2 plus NO^-_3 in nmol/mL.

Stastical Analysis:

Mann-Whitney U test was used to determine statistical significance between groups. The data are expressed as mean ± SD. A p value of less than 0.05 was considered stastically significant. Sensitivity, specificity and positive predictive value were calculated according to standard methods.

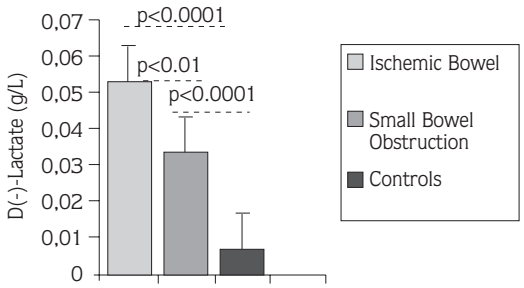

Figure 1. Serum D(-)-Lactate levels of ischemic bowel, small bowel obstruction and control groups.

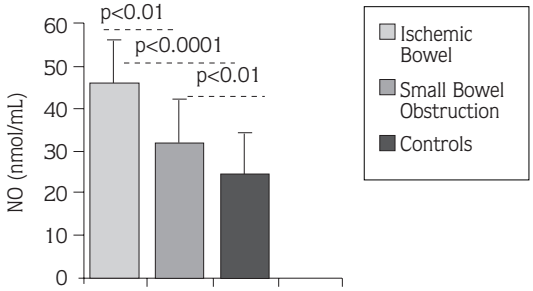

Figure 2. Serum NO levels of ischemic bowel, small bowel obstruction and control groups.

Results

Twenty-two patients underwent exploratory laparatomy. Mesenteric ischemia was found in twelve patients, of whom eight had small bowel infarction and four had small bowel and colonic infarction. The cause of ischemia was presumed to be embolic in twelve of these patients. Ten patients had a small bowell obstruction; that was related to adhesion. In the control group, patients underwent surgery for goitre but did not undergo exploratory laparatomy.

Serum D(-)-lactate levels of patients with ischemic bowel were found to be significantly increased compared to both the cases with small bowel obstruction and with control subjects ($p<0.01$, $p<0.0001$). Additionally, D(-)-lactate levels of small bowel obstruction cases were higher than those of the controls ($p<0.0001$) (Fig 1).

Patients with ischemic bowel had higher serum NO values than those of the controls and of small bowel obstruction ($p<0.0001$, $p<0.01$, respectively). NO levels of small bowel obstruction cases were higher than those of the controls ($p<0.01$) (Fig 2). There were significant correlations between serum NO and D(-)-lactate values both in mesenteric ischemic group and small bowel

obstruction group ($r=0.506$, $p<0.05$, and $r=0.692$, $p<0.05$, respectively).

Sensitivity is defined as the proportion of cases of mesenteric ischemia correctly diagnosed, and specificity as the proportion of diagnoses that mesenteric ischemias indeed absent in patients without the condition. For a D(-)-lactate level greater than 0.033 g/L, the sensitivity and the specificity of the D(-)-lactate assay were 75% and 90% respectively. For a NO level greater than 31 nmol/mL the sensitivity and the specificity of NO assay were 83% and 80% respectively. For a D(-)-lactate level greater than 0.033 g/L the positive predictive value was 81%. For a NO level greater than 31 nmol/mL, the positive predictive value was 71%.

Discussion

Until now, most of the reports related to lactic acidosis in human have referred only to L-lactic acidosis which is frequently associated with colitis, infectious diseases and malabsorption of carbohydrates. However, D-lactic asidosis was paid attension in recent years (17-19) and has been proposed that D(-)-lactate is produced by bacterial metabolism of unabsorbed carbohydrate in intestines. Mammalian tissues obey not produce D(-)-lactate and only slowly metabolizes it (6). Because of the resulting malabsorbtion of carbohydrates (19) large amounts of D- and L- lactate are produced by intestinal flora. With bacterial overgrowth, these metabolites are produced not only from mono- and disaccharides such as glucose and lactose (19,20) but also from starch, due to fermentative activity of certain abundant intestinal gram-positive rod-shaped bacteria (21) .

This study demonstrated that patients with mesenteric ischemia have significantly elevated D(-)-lactate levels in peripheral blood compared with patients who have small bowel obstruction and control groups. There are several reasons that D(-)-lactate might be a useful early predictor of acute mesenteric ischemia. Among these reasons, some conditions such as the overproduction of D(-)-lactate by bacteria in gastrointestinal tract, efflux of bacteria and the products of their metabolism including D(-)-lactate into the circulation due to injured mucosa seen in ischemic events, decrease in normal defense mechanism againts bacteria in intestinal ischemic conditions, and lack of enzyme systems rapidly metabolizing D(-)-lactate might be responsible.

Studies have implicated NO as a mediator, messenger, or regulator of cell function in physiologic states that include vascular tone, platelet function, septic shock (10). Masuda et al. (22) have demonstrated that endogenous NO may be an essential protective factor in the pathogenesis of ethanol-induced gastric mucosal injury through improved mucosal hemodynamics. There is an alternative approach to the explanation of the protective mechanism of NO that may function as a chemical barrier to cytotoxic free radicals because NO could be a natural extracellular scavenger of O2-. It seems that superoxide radicals possibly from endothelial cells themselves and/or Kupffer cells contribute the endothelial cell injury (9).

Because of its vasodilator effect it modulates tissue injury during the ischemia, and since it is a free radical it causes tissue injury by forming secondary toxic products. When tissue oxygen saturation is low it increases blood flow by vasodilatation and when tissue oxygen saturation is high it react with superoxide and forms toxic products such as peroxynitrite (13). In gastrointestinal system, evidence suggests that NO regulates mucosal blood flow, mucosal protection, hemodynamic responses to liver disease, hepatocyte synthetic function and relaxation of the muscularis (10). In an experimental study it had been showed that inhibition of endogenous NO formation with N-nitro-L-arginine (L-NNA), a selective NO synthase inhibitor, significantly aggregated macroscopic and microscopic mucosal injury caused by ethanol, and concurrent tratment with L-arginine, a precursor of NO, which competetively inhibits L-NNA actions significantly reduced the increased mucosal injury (22).

There are positive correlations between serum D(-)-lactate and NO levels in patients with acute mesenteric ischemia and small bowel obstruction. However, we could not explain this relation with the present knowledge.

As a result, since the diagnosis is very difficult with the clinics of acute mesenteric ischemic patients and routine laboratory findings, we believe that serum D(-)-lactate and NO measurements would be useful markers of acute intestinal ischemia.

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