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Original Article

The diagnostic efficacy of some ischemic markers (urotensin II and urotensin II related peptide) in early diagnosis of intestinal ischemia in experimentally increased intraabdominal pressure in rats

Mehmet Bugra Bozan[1], Refik Ayten[2], Nurullah Bulbuller[3], Cemalettin Camci[4], Erhan Aygen[2], Necip İlhan[5], Ibrahim Hanfi Ozercan[6], Ayse Azak Bozan[7], Ali Aksu[8], Nizamettin Kutluer[8]

[1]Kahramanmaras Sutcu Imam University, Faculty of Medicine, Department of General Surgery, Kahramanmaras, Turkey
[2]Firat University, Faculty of Medicine, Department of General Surgery, Elazig, Turkey
[3]Akdeniz University, Faculty of Medicine, Department of General Surgery, Antalya, Turkey
[4]Private Practice in General Surgery, Istanbul, Turkey
[5]Firat University, Faculty of Medicine, Department of Biochemistry, Elazig, Turkey
[6]Firat University, Faculty of Medicine, Department of Pathology, Elazig, Turkey
[7]Sehit Polis Fethi Sekin Training and Research Hospital, Department of Anesthesiology and Reanimation, Elazig, Turkey
[8]Sehit Polis Fethi Sekin Training and Research Hospital, Department of General Surgery, Elazig, Turkey

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Abstract

Aim: The purpose of this experimental study is evaluating the relation between plasma urotensin-II (U-II) and urotensin-II related peptide (URP) levels with intestinal ischemia caused by elevated intraabdominal pressure.

Material-Methods: Wistar albino rats weighing 200–250 grams used.

Group-1: Laparatomized group without abdominal pressure elevation in sixty minutes under general anesthesia.

Group-2: Ten mmHg elevated intraabdominal pressure group in sixty minutes under general anesthesia.

Group-3: Fifteen mmHg elevated intraabdominal pressure group in sixty minutes under general anesthesia.

Group-4: Twenty mmHg elevated intraabdominal pressure group in sixty minutes under general anesthesia.

After sacrifing, in general anesthesia blood and tissue samples were collected. Hematoxilen and eosyn stained tissue samples were examined under light microscope.

Results: Plasma U-II levels changes insignificant in group 2 and 3 but in group-4 was changed significantly compared with the control group(respectevely p=0,609; p=0.848; p=0.04). Plasma URP levels changed significantly in all groups compared with the control group (p-values were p=0.018; p=0.018 and p=0.04 respectively).

Conclusion: U-II levels could not be a useful parameter for the early diagnosis of the intestinal ischemic damage caused by intraabdominal pressure(IAP), because it is found in all vascular structures. On the other hand, plasma URP levels would be used for the early diagnosis of the intestinal ischemia. Because of the limitations of our study, we believe that our results should be confirmed with clinical studies.

Keywords: Intrabdominal hypertension; intestinal ischemia; urotensin-ii; urotensin-ii related peptid; abdominal compartment syndrome.

INTRODUCTION

Multiple organ dysfunction, which emerges because of the increase in the intraabdominal pressure (IAP), is also known as abdominal compartment syndrome (ACS) (1). The function loss in the cardiovascular, urinary and respiratory systems leads to multiple organ failure. A tense and distended abdomen, respiratory failure due to the increased airway pressure, hypoxia, hypercapnia, increased intraabdominal pressure, oliguria, and decrease in cardiac output (2), characterizes the clinical picture. It

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Corresponding Author: Mehmet Bugra Bozan, Kahramanmaras Sutcu Imam University, Faculty of Medicine, Department of General Surgery, Kahramanmaras, Turkey, **E-mail:** mbugrabozan@ksu.edu.tr

affects the stomach, duodenum, ileum, colon, and pancreas depending on the decrease of the mesenteric blood flow in the gastrointestinal system and the decrease of the blood flow in the ileal muscular layer. Its progression leads to ischemia, necrosis, and septic complications (1-3).

Urotensins are peptide hormones, which were first isolated from the caudal neurosecretory system of the rockfish in 1969 (4-6). Urotensin-II (U-II) and urotensin-II reseptors (UT) are secreted from various cells including the cells in the human brain, heart, kidney and tumor cells. They are used as markers in several cardiologic disorders (e.g. cardiomyopathy, coronary artery disease) (8).

In this study, our objective was to investigate the usability of urotensin II and urotensin II-related peptide (URP) in the early diagnosis of the intestinal ischemia in order to prevent the irreversible effects of the intestinal ischemia, which emerges due to the increase in the intraabdominal pressure, and the possible complications.

MATERIAL and METHODS

Preparation of the Subjects

In our study, we used Wistar Albino rats (200-250 g), which were obtained from the Experimental Research Center of Fırat University (FÜDAM) after the approval of the local Ethics Committee (Ethical approval number: 14/10/2009-26/64). The experiments were conducted in FÜDAM laboratory. All subjects were fed with the same standard food and city tap water in the same environment. The rats were fasted for 12 hours before the experiment.

Subject Groups

The rats were randomized into groups, which consisted of 7 rats each.

Group 1 (n=7) (control group):.The rats underwent only laparotomy and they were kept under general anesthesia for 60 minutes.

Group 2 (n=7): The rats were kept under general anesthesia for 60 minutes and the intraabdominal pressure was increased to 10 mmHg.

Group 3 (n=7): The rats were kept under general anesthesia for 60 minutes and the intraabdominal pressure was increased to 15 mmHg.

Group 4 (n=7): The rats were kept under general anesthesia for 60 minutes and the intraabdominal pressure was increased to 20 mmHg.

Anesthesia and Increase of Intraabdominal Pressure

The animals were anesthetized with intramuscular ketamine hydrochloride (50 mg/kg; Ketalar) and xylazine hydrochloride (5 mg/kg; Rompun). The abdominal wall was shaved and an antiseptic agent (10% povidone-iodine) was applied. Laparotomy was carried out with a midline incision under antiseptic conditions. In Group 1, general anesthesia and 1-cm incision laparotomy were implemented without creating an intraabdominal hypertension. In Group 2, following the administration of general anesthesia and a 2 cm incision for laparatomy, a Peritofix Catheter (B/Braun, Melsungen AG, Germany) was inserted into the intraperitoneal area for the pressure measurement and intraperitoneal 0.9% NaCl solution was infused to maintain a 10mmHg intraabdominal pressure for 60 minutes. IAP was measured with a monitored pressure transducer (Petaş, KMA 275, Turkey), which was connected to the catheter. A constant pressure of 10 mmHg was maintained with intermittent 0.9% NaCl infusions.In Group 3, the same method was applied but the IAP was adjusted to 15 mmHg with 0.9% NaCl infusions for 60 minutes. In Group 4, IAP was adjusted to 20 mmHg again with 0.9% NaCl infusions for 60 minutes. The additional need for anesthesia was provided with 50 mg/kg ketamine hydrochloride.

After surgery, the anesthetized rats were decapitated and in addition to blood samples, ileal tissue samples from approx. 2 cm proximal and distal to the ileocaecal valve and caecal tissue samples were obtained.The blood samples were centrifuged for 4 minutes (at 4000 rpm). The plasma and serum samples were stored at -200° C. The tissue samples were inserted into a 10% formaldehyde solution until the histological examination.

Evaluation of the Results

The study parameters Urotensin II and Urotensin II-related Peptide (URP) were measured in the Department of Medical Biochemistry in Medical School at Fırat University. Plasma levels of Urotensin II (Phoenix Pharmaceuticals Inc., USA, Catalogue No: EK-071-09), Urotensin II-related Peptide (Peninsula Laboratory LLC, USA, Catalogue No: S-1269) were analyzed with ELISA method in Biotek ELx50 (BioTek Instruments Inc., USA) (washing unit) and BioTek ELx800 (BioTek Instruments Inc., USA) (reading unit) devices.

Preparation of the Tissue Samples for the Histopathological Examination

A pathologist, who was blinded to the groups, prepared paraffin-embedded blocks from the ileum and caecum tissue samples, which were buffered in a 10% formaldehyde solution and sent to the pathology laboratory, and placed the cut 4-micrometer thick sections on the slides with positive ground edges in order to evaluate the findings of ischemia. The slides were stained with hematoxylin-eosin and were evaluated with magnifications of x10, x20 and x40 for the cellular changes. Histopathological examination was done with an Olympus BX51 light microscope in the Department of Medical Pathology in the Hospital of Fırat University.

For the evaluation of the intestinal damage caused by the increased abdominal pressure, we used the pathological classification, that was developed by Chiu et al. and modified by Yağmur et al., and scored accordingly (9,10) (Table 1).

Table 1. Modified Chiu Scoring System (9,10)

0:	Normal
1	Desquamation and necrosis of upper 1/3 villi
2	2Progressive peel off mid of villi
3	Peel off lower 1/3 villi and necrosis of cript cells
4	Necrosis of 2/3 cript cells
5	Complete loss of basal cripts

The pathological classification developed by Montgomery et al. was used for the ischemic scoring of the ischemic damage in the caecal tissue (11) (Table 2).

Statistical Analysis

The pathology results obtained in each group was done in U-II, URP, independent groups. The data, which obtained with the comparison of the pathological and biochemical results between the groups, were evaluated with ANOVA and Mann-Whitney U test. P values less than 0.05 were accepted as significant. The results were given in mean±standard deviation (SD). All analyses were done with SPSS for Windows v12.0 software package.

RESULTS

Biochemical Findings

U-II levels in the control group, 10-mmHg group, 15 mmHg group, and 20 mmHg groups were. 0.66±0.15 ng/ml;. 0.72±0.14 ng/ml;. 0.70±0.19 ng/ml and. 0.50±0.09. ng/ml respectively. There was no significant difference between the IAP groups and the control group except for 20 mmHg group (the comparison between the control group and 10 mmHg, 15 mmHg, and 20 mmHg groups revealed the p-values of p=.0.609.; p=.0.848., and p=0.04.respectively) (Table 3).

URP levels in the control group, 10 mmHg, 15 mmHg, and 20 mmHg groups were. 0.15±0.05. ng/ml;. 0.22±0.03 ng/ml;. 0.24±0.06 ng/ml, and. 0.07±0.04. ng/ml respectively. All groups had a significant difference compared to the control group (p-values were p=0.018; p=0.018 and p=0.04 respectively) (Table 3).

Table 2. Ischemic Injury Scoring System of Caecum (11)

0	Normal
1	Subepitelial eudema
2	Desqumation of superficial epitel
3	Upper 1/2 loss of glands
4	Loss of glands

Table 3. Biochemical levels of U-II and URP

Group	U-II (ng/ml)	URP (ng/ml)
Control	0.66 ± 0.15	0.15 ± 0.05
10 mmHg	0.72 ± 0.14	0.22 ± 0.03*
15 mmHg	0.70 ± 0.19	0.24 ± 0.06*
20 mmHg	0.50 ± 0.09*	0.07 ± 0.04*

* p<0.05 compared with control group

Table 4. Ischemic injury levels of ileum

Group	Modified Chiu Skoru
Control	0 ± 0
10 mmHg	0.85 ± 0.37*
15 mmHg	1 ± 0.57*
20 mmHg	2.85 ± 0.89*

* p<0.05 compared with control group

Table 5. Ischemic injury levels of caecum

Group	Ischemic Injury Scoring System of Caecum
Control	0 ± 0
10 mmHg	0.14 ± 0.37
15 mmHg	1.14 ± 0.69*
20 mmHg	1.85 ± 0.89*

* p<0.05 compared with control group

Pathological Findings

Pathological examination was performed with the tissue samples obtained from ileum and caecum as well as from the liver and kidney.

The examination of the ileal tissue samples revealed that there was a positive correlation between the increase in the intraabdominal pressure and ischemia level in the ileal segments. (the p-values in 10 mmHg, 15 mmHg and 20 mmHg groups compared to the control group were p=0.02; p=0.02; p=0.01 respectively). (Table 4). The same positive correlation was also observed in caecal tissue samples except for 10 mmHg group. (the p-values in 10 mmHg, 15 mmHg and 20 mmHg groups compared to the control group were p=0.317; p=0.03; p=0.02 respectively). (Table 5). The pathological appearance of the ischemic damage in the ileal tissue samples was shown in Figure 1, 2, 3 and 4 according to the groups.

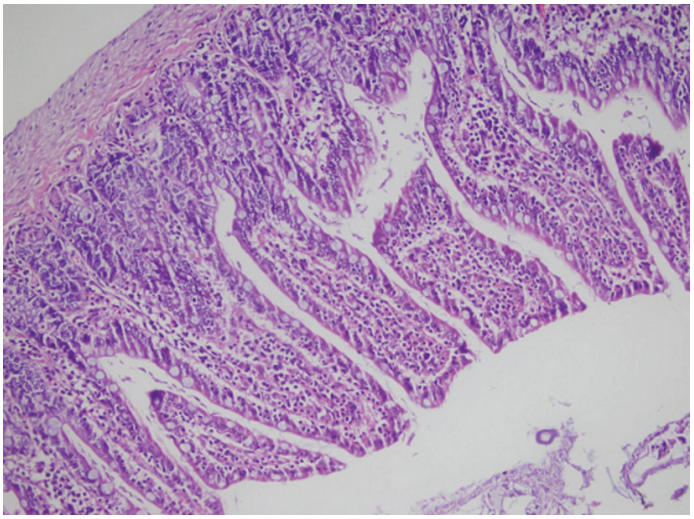

Figure 1. Pathologicalview of ileum in controlgroup, x20

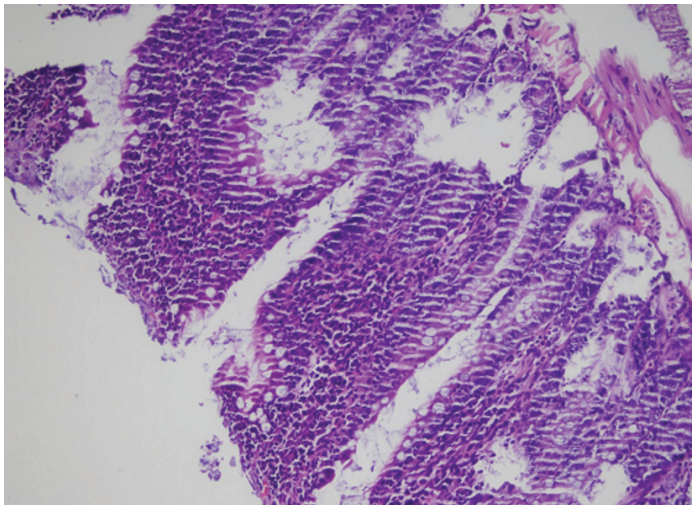

Figure 2. Pathological view of ileum under 10 mmHg abdominal pressure with desquamation formation, x20

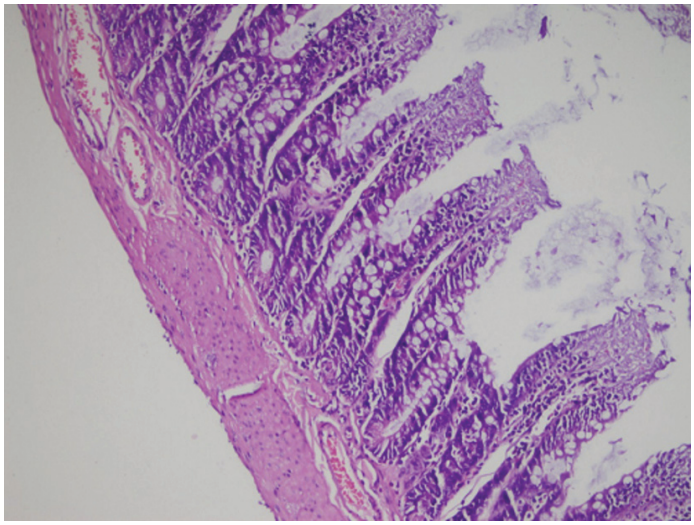

Figure 3. Pathological view of ileum under 15 mmHg abdominal pressure with progresive loss of villi, x20

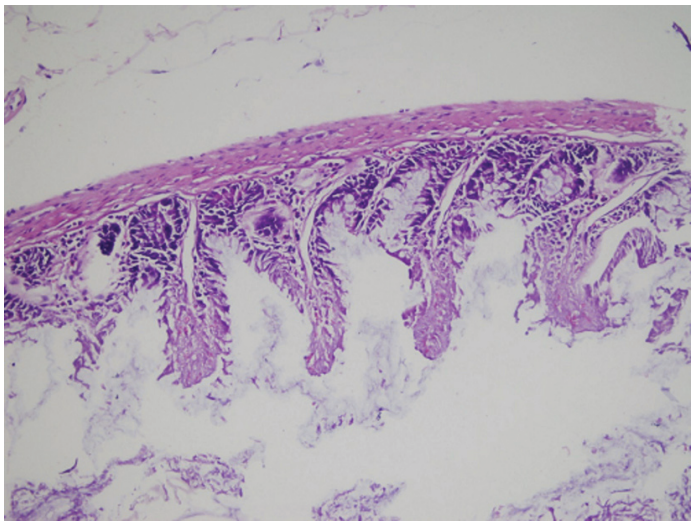

Figure 4. Pathological view of ileum under 20 mmHg abdominal pressure with necrozis in 2/3 of cript cells, x20

DISCUSSION

In spite of the experience on ACS extending over one century, the damage emerging after ACS maintains its importance. As it may develop also after surgical interventions, the surgeons should be aware of the importance of this syndrome and should take into consideration during their daily practice. The early diagnosis of this damage draws the attention of the investigators for years. Nevertheless, early diagnosis is critical in this syndrome, which has high morbidity and mortality rates.Richards and his colleagues were the first investigators, who described the development of kidney failure in patients with tense abdomen (12). Later, Kron et al. emphasized the importance of the decompression in the treatment of oliguria in patients with a bladder pressure over 25 mmHg and demonstrated that this process could be monitored with bladder catheterization at home (13). In the following studies focused on the abdominal compartment syndrome and its effects, it was reported that the multiple organ disorders, which developed after ACS, could be treated with abdominal decompression (1, 3).

The decrease in the mesenteric blood flow develops concomitant with the increase of the intraabdominal pressure. The pressure increase in the mesenteric vascular structures in addition to IAP causes a pseudo-occlusion, which leads to ischemia and necrosis and consequently to sepsis due to the bacterial translocation (14-16). Septic complications contribute also to the development of multiple organ damage (17, 18). Therefore, early diagnosis before the onset of irreversible damage is critical.

Human urotensin receptors function via cAMP and were described in several tissues (central nervous system, cardiovascular system, kidneys, bladder, prostate, liver, and adrenal glands). Furthermore, UT receptors are also synthesized in vascular smooth muscles, endothelium, and myocardium (8). U-II, which is a cyclic peptide molecule, is first isolated in fish tissues. Different types of this peptide hormonewas described in humans and it was considered as a potent vasoconstrictor agent found in the vascular structures of several mammalians. Consequently, the urotensinergic system and UT receptors are an important marker in abnormal vasoconstrictor diseases and diseases such as heart failure, pulmonary hypertension, which are characterized by cardiac dysfunction (8). Considering the presence of these receptors in multiple vascular structures, we believed that they would have a diagnostic value for the vasoconstriction caused by the IAP, but we did not observe any significant change in the U-II levels in subjects with an intraabdominal pressure of 10 mmHg and 15 mmHg (p -values. 0.609.and0.848, respectively). Only the comparison between 20 mmHg and control groups revealed a significant difference (p=0.04).

Urotensin II-related peptide (URP) was discovered in the year of 2003 and found out that it was an endogenous stimulator of the UT receptors (8). Taking the UT receptor activity into consideration, we compared the damage caused by IAP with the control group and observed a significant change in all groups (p-values in 10 mmHg, 15 mmHg and 20 mmHg groups were 0.018, 0.018, and 0.04 respectively) (Natural and synthetic peptides in the cardiovascular diseases: An update on diagnostic and therapeutic potentials).

However, as the blood levels of U-II and URP increase also depending on the activation of the renin-angiotensin-aldosterone system, it is not clear whether the changes in U-II levels depend on the renal damage or on the intestinal vascular vasoconstriction (7). Furthermore, IAP leads to a decrease in the cardiac outflow and cardiac pre-load and to a deterioration in the cardiac perfusion. Therefore, we could not conclude whether the changes in the U-II and URP levels were caused by the cardiac events or depended on the vasoconstriction and on the decrease of intestinal blood flow due to the IAP (8,19).

CONCLUSION

We concluded that U-II could not be a useful parameter

for the early diagnosis of the intestinal ischemic damage caused by AIP, because it is found in all vascular structures. On the other hand, plasma URP levels could be used for the early diagnosis of the intestinal ischemia. Because of the limitations of our study, we believe that our results should be confirmed with clinical studies.

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Mehmet Bugra Bozan ORCID: 0000-0001-5573-2645
Refik Ayten ORCID: 0000-0001-8794-0449
Nurullah Bulbuller ORCID: 0000-0001-6126-0808
Cemalettin Camci ORCID: 0000-0002-0908-4971
Erhan Aygen ORCID: 0000-0003-4481-480X
Necip Ilhan ORCID: 0000-0001-9997-0418
Ibrahim Hanefi Ozercan ORCID: 0000-0002-2971-3536
Ayse Azak Bozan ORCID: 0000-0001-8737-4408
Ali Aksu ORCID: 0000-0002-9226-1720
Nizamettin Kutluer ORCID: 0000-0002-1092-2979

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