Ulus Travma Acil Cerrahi Derg 2010;16 (2):103-107

Renal cytokine and histopathologic changes following acutely increased intra-abdominal pressure: an animal study

Karın içi basıncın hızla artışı sonrası gelişen renal histopatolojik ve sitokin değişiklikleri: Bir hayvan çalışması

Gökhan AKBULUT,[1] Mustafa ALTINDİŞ,[1] Fatma AKTEPE,[1] Mustafa SERTESER,[2] Osman Nuri DİLEK[3]

BACKGROUND

The reason for acute renal failure (ARF) in abdominal compartment syndrome (ACS) is thought to be due to the increase in renal venous pressure. In this study, the changes in plasma and renal tissue cytokine levels and histopathologic changes in renal tubular and glomerular cells were evaluated and compared in a model of acute elevation in abdominal tension with different pressures.

METHODS

Eighteen Sprague-Dawley rats were randomly assigned into three groups: Sham-operated rats and rats in Groups 1 and 2, in which 20 and 30 mmHg of intra-abdominal pressure (IAP) was applied for 60 minutes, respectively. Left kidneys of the animals and intracardiac blood samples were taken at the end of the procedures. Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) levels were investigated in plasma and renal tissue samples. Renal tissues were also evaluated for histopathologic changes.

RESULTS

Renal tissue and plasma TNF-α and IL-6 levels were found to be significantly increased when the sham-operated group was compared to the study groups ($p<0.05$). Renal tissue changes showed that the total histopathologic scores were significantly increased in study groups compared to the sham-operated group ($p<0.05$); tubular changes were more prominent than glomerular changes.

CONCLUSION

Abdominal tension linearly can cause renal tubular histopathologic changes. Cytokines may play a role in ARF due to ACS.

Key Words: Acute renal failure; cytokine; histopathology; intra-abdominal pressure; oliguria; trauma.

AMAÇ

Abdominal kompartman sendromu sonrası gelişen akut böbrek yetersizliğinin renal venöz basıncın artışı ile ilişkili olduğu düşünülmektedir. Bu çalışmada, değişik karın içi basınç değerlerinde renal tübüler ve glomerüler hücrelerdeki histopatolojik değişiklikler ile plazma ve renal doku sitokin düzeylerindeki değişiklikler araştırıldı ve karşılaştırıldı.

GEREÇ VE YÖNTEM

On sekiz Spraque-Dawley cinsi sıçan rastgele üç gruba ayrıldı: Sham grubu, basıncın 20 ve 30 mmHg düzeyinde 60 dakika tutulduğu grup 2 ve grup 3. Deneklerin, sol böbrekleri ve intrakardiyak kan örnekleri işlem sonunda alındı. Tümör nekroz faktör alfa (TNF-α) ve interlökin 6 (IL-6) düzeyleri hem renal dokuda hem de plazmada araştırıldı. Renal dokular, histopatolojik değişiklikler açısından da değerlendirildi.

BULGULAR

Sham grubu çalışma grupları ile karşılaştırıldığında, renal doku ve plazma TNF-α, IL-6 düzeylerinin anlamlı olarak arttığı saptandı ($p<0,05$). Renal doku değişiklikleri değerlendirildiğinde, total histopatolojik skorların arttığı ($p<0,05$) tübüler değişikliklerin, glomerüler değişikliklerden daha belirgin olduğu saptandı.

SONUÇ

Sonuç olarak, karın içi basınç artışı, doğrusal olarak renal tübüler histopatolojik değişiklikleri arttırmaktadır. Sitokinler abdominal kompartman sendromunda gelişen akut böbrek yetersizliğinde rol oynuyor olabilirler.

Anahtar Sözcükler: Akut böbrek yetersizliği; sitokin; histopatoloji; karın içi basınç; oligüri; travma.

[1]Dept.'s of General Surgery, Microbiology and Pathology, Afyon Kocatepe University Faculty of Medicine, Afyonkarahisar; [2]Dept. of Biochemistry, Acıbadem University Faculty of Medicine, Istanbul; [3]Dept. of General Surgery, Sakarya University Faculty of Medicine, Sakarya, Turkey.

[1]Afyon Kocatepe Üniversitesi Tıp Fakültesi Genel Cerrahi, Mikrobiyoloji, Patoloji Anabilim Dalları, Afyonkarahisar; [2]Acıbadem Üniversitesi Tıp Fakültesi Biyokimya Anabilim Dalı, İstanbul; [3]Sakarya Üniversitesi Tıp Fakültesi Genel Cerrahi Anabilim Dalı, Sakarya.

Correspondence *(İletişim):* Gökhan Akbulut, M.D. Ali Çetinkaya Kampüsü, Mavi Hastane, A Blok Kat 2, 03200 Afyonkarahisar, Turkey.
Tel: +90 - 272 - 246 33 33 Fax *(Faks):* +90 - 272 - 246 33 22 e-mail *(e-posta):* akbulutg@asia.com

The abdominal compartment syndrome (ACS) is defined as the adverse physiologic consequences that occur as a result of an acute increase in intra-abdominal pressure (IAP).[1] One of those adverse consequences is acute renal failure (ARF) that is unresponsive to resuscitation. Renal derangements of acute increase in IAP are thought to be multifactorial, including decreased cardiac output and direct renal parenchymal and renal venous compression.[1] Cytokines could play a role in renal failure due to acute elevations in IAP. To our knowledge, there is no study in the literature showing the changes in renal and plasma cytokine levels associated with histopathologic parameters in acute elevation of IAP using different pressures.

This study aimed to evaluate plasma and renal tissue cytokine changes and the changes in histopathologic parameters of renal tissue after acute elevation in IAP using different pressures.

MATERIALS AND METHODS

All procedures mentioned were approved by the local ethical authority. The study protocol was designed in accordance with the 1996 revised form of the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health. Unnecessary animal suffering was avoided throughout the study.

The study included 18 adult female specific pathogen-free Sprague-Dawley rats (200-220 g), isolated from male rats, purchased from Selcuk University, Experimental Research Laboratories (Konya, Turkey). Animals were housed in standard laboratory cages and were allowed free access to food and water until 12 hours before the surgical procedures.

Experimental Design

This study was designed as an experimental, randomized, controlled trial with blind assessment of outcome. The rats were randomly assigned into the three groups as follows: Sham-operated group (n=6: Rats underwent the same surgical procedure except for increasing the abdominal pressure. Group 1 (n=6): IAP was increased to 20 mmHg for 60 min. Group 2 (n=6): IAP was increased to 30 mmHg for 60 min.

Surgical Procedure

The rats were anesthetized with 100 mg/kg ketamine (Ketalar™ amp, EIP, Istanbul, Turkey). A rectal probe was placed to monitor the body temperature. A heating pad was maintained until animals recovered from anesthesia to keep the body temperature at 37°C. Under aseptic conditions, a 1-cm-long midline abdominal incision was made. A physiologic saline-filled gastric tube was inserted into the stomach orally and its position was checked manually. One mL of physiologic saline was injected into the stomach and the other end of the gastric tube connected to a linear manometer to check IAP indirectly. A sterile balloon connected to a tube was inserted totally into the abdomen. The other end of the tube was connected to the insufflator (Karl Storz™, Model no: 26-020 S, Insufflator, West Germany). Room air was used to insufflate the balloon at room temperature. Each time, at the end of the surgical procedure, balloons were checked for penetration. The balloon in the abdomen was insufflated until the IAP reached the level of 20 mmHg in Group 1 and 30 mmHg in Group 2. IAP was stabilized at 20 mmHg and 30 mmHg in Groups 1 and 2, respectively, and maintained for 60 min in both study groups. At the end of the surgical procedure, laparotomy incision was performed and the balloon was removed and checked for penetration for gas efflux. If there was no efflux or penetration, the left kidney was removed and divided into two parts on its axis for histopathologic and tissue cytokine analysis. One part of the kidney was stored in 10% formaldehyde solution, and the other was washed three times with physiologic saline and stored at -20ºC for cytokine analysis. Then, intracardiac blood samples were taken on EDTA-containing tubes, and rats were euthanized by hypovolemic shock under general anesthesia. Blood samples were centrifuged at 3000 g, +4ºC for 5 min and plasma samples were removed and stored at -20ºC for cytokine analysis.

A code number was given to each kidney and blood sample, and further analysis was performed in a blinded fashion.

Histopathologic Analysis

Kidney samples were placed into buffered formalin solution. After 24 hours, they were processed and embedded in paraffin. Histopathologic sections were stained with hematoxylin and eosin and assessed under light microscope. All sections were reviewed by the same pathologist who was blinded to the study group allocations. Histopathologic changes in proximal tubules were scored from 0 to 3 for each tubular profile: 0= normal histology; 1= tubular cell swelling, brush border loss, nuclear condensation, nuclear pyknosis, cytoplasmic eosinophilia, shedding of the lining cells, and complete loss of the lining cells, with up to 1/3 of tubular profile; 2= same as for score 1, but greater than 1/3 and less than 2/3 of tubular profile; 3= greater than 2/3 of tubular profile.[2]

Tissue Homogenization

A portion of each renal tissue was homogenized for all assays. Homogenization was performed in 1:10 (w:v) 0.1 M potassium phosphate buffer (pH=7.4) with a Ultra Turrax homogenizer (IKA T18 Basic™, Wilmington, NC, USA). After centrifugation of the homogenates at 10000 rpm at +4°C for 10 min, the supernatants were removed and subjected to analysis.

Table 1. Renal tissue and plasma tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) levels between groups (data are expressed as mean±standard error of mean and picogram per deciliter [pg/dL])

Group	Renal TNF-alpha (pg/mg protein)	Renal IL-6 (pg/mg protein)	Plasma TNF-alpha (pg/mL)	Plasma IL-6 (pg/mL)
Sham	9711.3±623.4	2765.5±273.3	5395.6±195.5	865.8±36.7
Group 1	16765.80725.87*	3918.83161.33	13417.80282.76*	1204.3348.87*
Group 2	24702.501354.64*	7438.67395.51*	53382.86 2026.06*	1892.3384.70*

* p<0.01 sham vs Group 1 and Group 2; # p<0.05 sham vs Group 1; p<0.05 Group 1 vs Group 2.

Table 2. Renal tissue histopathologic changes (all changes scored from 0 to 3) (data are expressed as mean±standard error of mean; Min: minimum, Max: maximum)

Group		Swelling	Brush border loss	Pyknosis	Cytoplasmic eosinophilia	Complete loss of the lining cells	Shedding of the lining cells	Total
Sham	Mean±SEM	0.3±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.2
	Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Max	1.0	0.0	0.0	0.0	0.0	0.0	1.0
Group 1	Mean±SEM	1.3±0.2*	1.0±0.0*	0.5±0.2	0.5±0.2	0.0±0.0	0.0±0.0	3.3±0.4*
	Min	1.0	1.0	0.0	0.0	0.0	0.0	2.0
	Max	2.0	1.0	1.0	1.0	0.0	0.0	5.0
Group 2	Mean±SEM	1.5±0.1**	1.8±0.2*	0.2±0.2	0.2±0.2	0.0±0.0	0.0±0.0	3.8±0.3*
	Min	1.0	1.0	0.0	0.0	0.0	0.0	3.0
	Max	2.0	2.0	1.0	1.0	0.0	0.0	5.0

* p<0.01 vs sham-operated rats; ** p<0.05 vs sham-operated rats.

Immunologic Analysis

Interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha levels were determined using a commercially available rat ELISA kit (Biosource Int., USA), and the results were expressed as picograms per milligram (pg/mg) protein for tissue samples and picogram per milliliter (pg/mL) for plasma samples. Tissue protein levels were determined by Biuret method.

Statistical Analysis

The results were decoded and statistically analyzed. Data were expressed as the mean±standard error of the mean (SEM). Statistical analysis was performed using Kruskal-Wallis and Mann-Whitney U test. A p value <0.05 was considered to be significant. Data were analyzed with SPSS for Windows software version 10.0 (SPSS, Inc., Chicago, Illinois).

RESULTS

Results are summarized in Tables 1 and 2. Renal tissue and plasma TNF-α and IL-6 levels were significantly increased when the sham-operated group was compared to Group 1 (p<0.05 for renal tissue IL-6, p<0.01 for other parameters). Renal tissue and plasma TNF-α and IL-6 levels were also found to be increased when the sham-operated group was compared

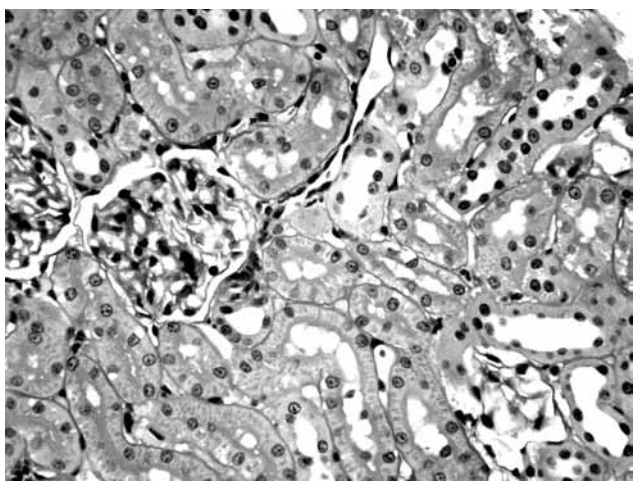

Fig. 1. Normal histologic appearance in the renal tissue of the control group (H-E x 400).

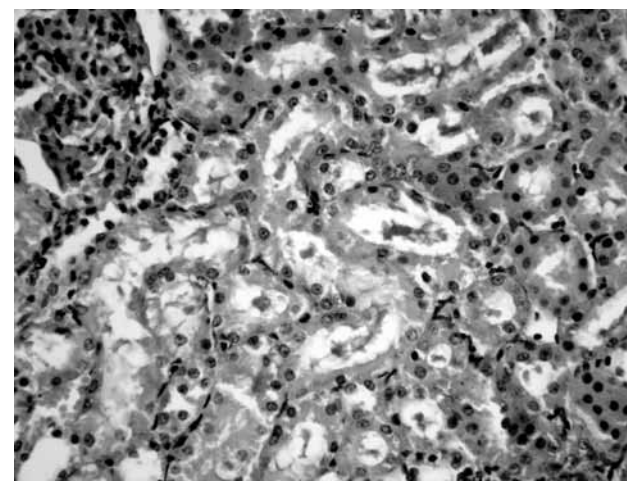

Fig. 2. Histopathologic appearance of the renal tissue of Group 1. Proximal tubules show swelling and brush border loss of the lining cells (H-E x 400).

to Group 2 ($p<0.01$) and when Group 1 was compared to Group 2 ($p<0.01$).

When renal tissue changes were evaluated, the total histopathologic scores and the scores of swelling and brush border loss of renal tubular cells were significantly increased in the two study groups when compared to the sham-operated group ($p<0.05$) (Figures 1 and 2). No changes were observed between Groups 1 and 2, in glomerular cells.

DISCUSSION

ACS causes significant derangements in renal function. The rapid elevation in IAP is associated with oliguria followed by anuria with the progression of renal failure.[3-7]

Renal failure caused by ACS has been thoroughly documented both clinically and experimentally. Harman et al.[8] reported the results of a canine study in which IAP was increased from 0 to 40 mmHg while hemodynamic parameters and renal function were monitored. Cardiac output (CO), renal perfusion and urinary output were all found to be decreased as IAP was increased. At 40 mmHg, dogs were resuscitated and CO returned to normal; however, neither renal blood flow nor glomerular filtration rate returned to normal; this was accomplished only with abdominal decompression. The authors also placed stents into the ureters of two animals to determine whether ureteral compression played a role in the mechanism of oliguria. The ureteral stents had no effects. The authors concluded that the oliguria due to ACS was related to direct compression of the kidney.

Doty et al.[9] investigated the isolated effects of elevation of renal parenchymal pressure on renal function. They used a compressive device consisting of two acrylic plates transversed by four screws placed beyond the confines of the kidney. The screws were tightened and increasing renal parenchymal pressure was measured with a needle connected to a pressure transducer placed approximately 1 cm deep into the lateral border of the kidney without changing systemic hemodynamic parameters. After isolated renal parenchymal pressure increased to the level of 30 mmHg, they found no renal hemodynamic changes or hormonal changes.

Those expertly designed experimental studies showed that the reasons for renal failure in acute elevation of IAP is sophisticated; except for renal venous compression, neither parenchymal or ureteral compression, nor changes in systemic hemodynamic parameters alone caused renal derangements.[10] In those sophisticated conditions, inflammation, which is orchestrated by cytokines, may play a role in renal failure in ACS. TNF-α and IL-6 are among the main and potent pro-inflammatory cytokines.[11-13] We proposed that cytokines may play a role in renal derangements in ACS. We found renal tissue and plasma cytokine levels significantly increased after acute elevation in IAP. These changes were associated with histopathologic changes in renal tubular cells. We highlight two points: first, in this study, inflammation was triggered in acute elevation of IAP, but in higher pressures, the levels of renal and plasma TNF-α and IL-6 were not significantly increased. Second, this does not explain why renal tubular cells are affected or why changes are more prominent than in glomerular cells. The available findings in the literature do not satisfactorily explain those results.

In the present study, swelling and erasing of the brush border of tubular cells were observed in both study groups compared to the sham-operated rats, but no histopathologic changes were observed between study groups. Doty et al.[10] showed that isolated elevation in renal venous pressure reduces glomerular filtration and renal blood flow and promotes release of renin and aldosterone levels. However, in our study, the histopathologic changes in renal tubular cells were more prominent than in glomerular cells. If renal derangements are caused by elevation in renal venous pressure throughout the glomerular filtration, glomerular histopathologic changes should be more prominent. Further studies are needed to understand the mechanism of the renal tubular changes in ACS.

In conclusion, acute elevation in IAP causes an increase in plasma and renal tissue cytokine levels with associated histopathologic changes in renal tubular cells. Increase in cytokine levels continues if IAP reaches higher levels.

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