



The role of cobalt-albumin binding analysis in the diagnosis of experimental abdominal compartment syndrome in rabbits

Tavşanlarda deneysel abdominal kompartman sendromunun tanısında kobalt-albümin bağlanma analizinin rolü

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BACKGROUND

The purpose of our study was to examine the role of cobalt-albumin binding assay (CABA) for the early diagnosis of abdominal compartment syndrome (ACS).

METHODS

Twenty-four anesthetized and ventilated rabbits were randomly assigned to four groups as 1 to 4, with each group comprised of six animals. Intraabdominal hypertension of 25 mmHg was induced for 15, 30, 45, and 60 minutes by insufflation in the four groups, respectively. Five ml of blood was drawn from each animal before the animals were sacrificed. A CABA test was performed on the samples and results were compared with pathologic diagnosis of intestinal samples shown as a score of damage severity values.

RESULTS

Ischemia-modified albumin (IMA) in Group 4 was significantly higher than in Group 1 and Group 2 (0.65 ± 0.16 , 0.60 ± 0.25 and 0.61 ± 0.14 , respectively; $p<0.05$). However, there was no significant difference between the IMA of Group 3 and Group 4. Score of damage severity values reached statistically significant levels in Group 4 compared with Group 1 and Group 2 ($p<0.004$ and 0.006 , respectively) and in Group 3 compared with Group 1 ($p<0.004$). There was also a statistically significant difference between Groups 1 and 2 ($p<0.004$).

CONCLUSION

CABA plays an important role in the early diagnosis of ACS at the beginning of intestinal ischemia.

Key Words: Abdominal compartment syndrome; ischemia-modified albumin; rabbit.

AMAÇ

Çalışmanın amacı, abdominal kompartman sendromunun (AKS) erken tanısında kobalt-albümin bağlanma testinin (KABT) rolünü incelemektir.

GEREÇ VE YÖNTEM

Her grupta altı hayvan olmak üzere, 24 anestezi uygulanmış ve ventile edilen tavşan rastgele dört gruba ayrıldı. 25 mmHg karın içi hipertansiyon gaz verme yoluyla, sırasıyla 15., 30., 45. ve 60. dakikalarda elde edildi. Hayvanlar öldürülmeden önce her birinden 5 mL kan örneği alındı. Örnekler üzerinde KABT testi uygulandı ve hasar şiddet skoru olarak gösterilen bağırsak örneklerinin patolojik tanıları ile karşılaştırıldı.

BULGULAR

Dördüncü gruptaki iskemi modifiye albümin (İMA) hem Grup 1 hem de Grup 2'den istatistiksel olarak daha büyük bulundu (sırasıyla $0,65\pm 0,16$, $0,60\pm 0,25$ ve $0,61\pm 0,14$) ($p<0,05$). Grup 3 ve Grup 4'ün İMA değerleri arasında istatistiksel bir fark bulunmadı. Grup 4, Grup 1 ve Grup 2 ile karşılaştırıldığında (sırasıyla $p<0,004$ and $0,006$) ve Grup 3, Grup 1 ile karşılaştırıldığında hasar şiddet skoru istatistiksel olarak anlamlı bulundu ($p<0,004$). Grup 1 ve Grup 2 arasında da istatistiksel olarak anlamlı fark bulundu ($p<0,004$).

SONUÇ

AKS'nin erken tanısında iskeminin başlangıcında KABT önemli bir rol oynamaktadır.

Anahtar Sözcükler: Abdominal kompartman sendromu; iskemi modifiye albümin; tavşan.

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Abdominal compartment syndrome (ACS) has been broadly defined as organ dysfunction attributed to increased intra-abdominal pressure (IAP). The effects of ACS on abdominal contents and the respiratory system were of major interest to early investigators.^[1-3] In 1984, the first case series in which IAP measurement was used as a criterion for abdominal decompression was reported by Kron et al.,^[4] who were the first to use the phrase ACS. A correlation between increased IAP and decrease in cardiac output, total lung capacity, and functional residual capacity was well defined in animal models.^[5,6] Furthermore, with advances in laparoscopic procedures, many investigators attempted to demonstrate altered hemodynamics associated with increased IAP in humans.^[7,8]

Markedly increased IAP occurs widely after extensive abdominal trauma. Many factors contribute to this phenomenon, such as accumulation of blood and clots and bowel edema or congestion resulting from injury to mesenteric vessels. Diabel et al.^[9,10] showed that increased IAP could cause severe intestinal ischemia and significant decreases in mesenteric artery blood flow, although cardiac output and mean arterial pressure (MAP) remained normal. The investigators also showed that continued IAP of 20-25 mmHg for 60 minutes (min) caused deterioration of intestinal-barrier function.^[11] Thus, the early detection of increased IAP and its management are particularly important for decreasing the morbidity and mortality of patients.

Human serum albumin is a blood protein with a cobalt-binding site at the N-terminus. Studies have hypothesized that ischemic tissue potentially alters the N-terminus, rendering it incapable of binding cobalt.^[12-14] Human serum albumin that cannot bind cobalt because of an ongoing ischemic event is referred to as ischemia-modified albumin (IMA).^[14] The quantification of IMA (Inverness Medical Professional Diagnostics, Princeton, NJ, US) is a United States Food and Drug Administration-approved assay currently used in many emergency room settings. Numerous clinical and animal studies have been conducted that demonstrate a correlation between a positive cobalt-albumin binding assay (CABA) test, ischemia, acute coronary syndrome, and trauma.^[15,16] IMA is established as a sensitive marker of skeletal muscle ischemia, pulmonary embolism and stroke.^[17-22] Currently, to our knowledge, there are no published data or studies showing whether IMA increases in ACS.

The purpose of this experimental study was to evaluate the changes in IMA levels occurring during CO₂ pneumoperitoneum (PNM) at a stable IAP level for various time durations at 15-min intervals. We also aimed to look for a possible ischemic injury of the small intestine by pathologic examination to clarify the usage of IMA as a diagnostic test for early detection of ABC.

MATERIALS AND METHODS

The study protocol was approved by the Animal Research Committee of Dokuz Eylul University. Twenty-four female New Zealand White rabbits weighing between 2400 and 3700 g were used in this study. The rabbits were fasted overnight but were allowed access to water.

Anesthesia and Monitoring of Animals

All rabbits were anesthetized with intramuscular injections of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg). The vein of the right ear was cannulated and an infusion of 0.9% NaCl solution was started at a rate of 4 ml/kg/h. The artery of the left ear was also cannulated for monitoring arterial blood pressure and arterial blood gas sampling. The invasive arterial blood pressures were followed throughout the experiments on the monitor (PETAS KMA 450 Profesyonel Elektronik A.Ş., Ankara, Turkey). A tracheotomy was performed and a cannula (Portex tracheal tube 3 mm ID; Portex-SIMS, Portex Ltd, Hythe, UK) was inserted into the trachea.

After a bolus administration of 0.6 mg/kg rocuronium bromide, the rabbit was ventilated with oxygen-supplemented room air using a volume-cycled ventilator (Evita 2; Drägerwerk AG, Lübeck, Germany), which was adjusted for maintaining normocapnia at a respiration rate of 20/min, tidal volume 12-15 ml/kg. The end-tidal pressure of carbon dioxide (PETCO₂) was continuously measured with a capnograph (Anesthesia Gas Monitoring 1304®, Brüel & Kjaer, Copenhagen, Denmark) and maintained in the range of 35-40 mmHg.

The body temperature of the animals was monitored with a rectal temperature probe during the experiments; it was regulated with a servomechanism at 37.0±0.1 °C with a heat lamp. During the surgical preparation, anesthesia was maintained with repeated intravenous injections of ketamine/xylazine (10 mg/kg-1/2 mg/kg; IV) administered IV as necessary to maintain the depth of anesthesia. Appropriate depth was determined by lack of a righting reflex and by testing the palpebral and pedal withdrawal reflexes every 10 min as previously described by Wyatt et al.^[23] All experiments were performed under the same conditions.

Surgical Procedures

The animals were fixed to a surgical table in a supine position. After steady state had been reached, a Veress needle (U.S. Surgical Corporation, Norwalk, CT, US) was placed into the peritoneal cavity. After iatrogenic puncture risk was eliminated, 3 ml NaCl was injected through the Veress needle to verify the intra-abdominal placement of the needle.^[24] Insufflation with CO₂ was performed using an Op-Pneu insufflator (Wisap, Sauerlach, Germany) at a rate of 2-3 L/

min until the IAP reached 25 mmHg. At the end of the experiments, all of the animals were sacrificed.

Experimental Protocol

The animals were randomly divided into four groups, each consisting of six rabbits according to the duration for which a level of 25 mmHg IAP was maintained. In Group 1, CO₂ PNM was maintained at an IAP of 25 mmHg for 15 min; in Group 2, CO₂ PNM was maintained at an IAP of 25 mmHg for 30 min; in Group 3, CO₂ PNM was maintained at an IAP of 25 mmHg for 45 min; and in Group 4, CO₂ PNM was maintained at an IAP of 25 mmHg for 60 min.

Monitoring the Physiological Parameters

The heart rate, MAP, PETCO₂, and rectal temperature were continuously monitored and recorded every 15 min. For arterial blood gas analysis (ABL 700 Series; Radiometer Copenhagen, Copenhagen, Denmark), pressure of arterial carbon dioxide (PaCO₂), pressure of arterial oxygen (PaO₂), pH, and base excess were measured. All parameters were recorded before CO₂ PNM and at 15-min intervals after establishing the CO₂ PNM.

Determination of IMA Levels

Five ml blood samples for IMA and albumin measurement were taken from peripheral veins of the animals from Groups 1, 2, 3, 4 at 15 min, 30 min, 45 min, and 60 min of CO₂ PNM, respectively. After sampling for IMA, the animals were sacrificed. Samples remained for 1 hour (h) at room temperature until they clotted. Then samples were centrifuged for 10 min at 4000 rpm to obtain serum specimens. Serum specimens were stored at +4 °C and analyzed within 24 h.

Ischemia-modified albumin (IMA) levels were measured according to Bar-Or et al.'s colorimetric method.^[14] Fifty µL of 0.1% CoCl₂ (Sigma-Aldrich Lot: S38901-248 Cat: 20,218.5) was added to 200 µL serum samples, mixed vigorously and incubated for 10 min for cobalt-albumin binding. Then, 50 µL DTT (1.5 mg/dl) (Sigma-Aldrich Lot: D5545-1G Cat: 117K0663) was added as a coloring agent. After 2 min, 1 ml 0.9% NaCl was added to halt the binding between the cobalt and albumin. Using a spectrophotometer at 470 nm (Shimadzu, model UV-1201V), color development with DTT was compared to a serum-cobalt blank without DTT and reported in absorbance units (ABSU). Albumin measurements were performed according to bromocresol green method by Architect C16000 (Abbott Diagnostic, IL, US) auto analyzer.

Histopathologic Analysis

The animals were sacrificed after the procedures, and the intestine was removed and fixed in 10% formalin for 24 h and then transferred to 70% ethanol.

After graded dehydration, tissue samples were embedded in paraffin wax, sectioned transversely at 4 micrometers, stained with hematoxylin and eosin (H-E), and examined with a light microscope. H-E-stained slides were evaluated by light microscopy by pathologists (EV-UB) who were not informed of the experimental conditions for any given specimen.

A semi-quantitative histological assessment of intestinal injury was utilized to obtain an overall score of damage severity in all regions of the small bowel sampled. A total score for each region of the intestine was derived from the sum of scores for 11 histological criteria.^[25] According to this study, these criteria were villus fusion and stunting (atrophy), disruption of brush border and surface enterocytes, reduction in goblet cell number, reduction in numbers of mitotic figures, crypt loss/architectural disruption, disruption or distortion of crypt cells, crypt abscess formation, infiltration of polymorphonuclear cells and lymphocytes, and dilatation of lymphatics and capillaries, together with thickening and edema of the submucosal and muscularis external layers. Each histological variable was scored by pathologists from 0 (normal) to 3 (maximal damage) to give a maximum possible score of 33 for each intestinal region.

Statistical Analysis

Statistical analysis was carried out using SPSS® for Windows v. 15.0 (SSPS, Inc., Chicago, IL, US). Data from all groups were expressed as means ± standard deviation. The difference among groups was assessed with one-way ANOVA and Tukey HSD test to examine the differences between two groups. $p < 0.05$ was considered as statistically significant.

RESULTS

Heart rate, MAP, PETCO₂, pH, PaCO₂, PaO₂, and lactate levels did not change over time in any of the groups (Table 1).

Ischemia-modified albumin (IMA) levels were significantly increased in Group 4 compared to Group 1 and Group 2 (0.65 ± 0.16 , 0.60 ± 0.25 and 0.61 ± 0.14 , respectively; $p < 0.05$). However, there were no statistically significant differences between Group 3 and Group 4 (Fig. 1). The albumin concentrations showed no difference between any of the groups ($p > 0.05$). Intestinal injury score was significantly increased in Group 4 compared to Group 1 and Group 2 ($p = 0.004$ and $p = 0.006$, respectively). Also, intestinal injury score was significantly increased in Group 3 compared to Group 1 ($p = 0.04$) and Group 1 compared with Group 2 ($p = 0.004$). However, there were no statistically significant differences between Group 2 and Group 3 or between Group 3 and Group 4 in intestinal injury score (Fig. 2).

Table 1. Physiological variables

Variable	Group 1 (15 min)	Group 2 (30 min)	Group 3 (45 min)	Group 4 (60 min)
Heart rate (beats/min)				
Baseline	200.33±17.91	202.67±13.55	206.33±17.36	182.00±24.49
15 min.	200.00±10.12	210.00±6.57	200.00±14.53	194.00±15.95
30 min.		206.00±10.95	204.00±13.15	186.00±22.45
45 min.			200.00±9	193.00±15.27
60 min				182.67±23.69
MAP (mmHg)				
Baseline	65.06±10.05	72.66±7.65	80.66±15.77	67.21±11.68
15 min.	62.59±9.32	67.33±10.26	61.88±12.89	66.71±5.38
30 min.		57.60±17.20	58.94±10.92	62.22±14.58
45 min.			58.16±11.87	56.99±7.93
60 min				67.49±8.30
Arterial pH				
Baseline	7.46±0.12	7.46±0.07	7.49±0.05	7.48±0.11
15 min.	7.53±0.13			
30 min.		7.49±0.05		
45 min.			7.53±0.03	
60 min.				7.53±0.03
PaCO₂ (mmHg)				
Baseline	37.06±2.80	35.81±1.87	34.88±1.81	33.10±1.69
15 min.	36.30±6.62			
30 min.		34.43±4.01		
45 min.			34.70±3.36	
60 min				30.48±0.94
PaO₂ (mmHg)				
Baseline	253.53±92.60	313.98±75.78	238.90±41.61	322.28±103.52
15 min.	290.42±170.75			
30 min.		324.65±92.30		
45 min.			252.40±107.66	
60 min				265.88±53.05
PETCO₂ (mmHg)				
Baseline	38.08±1.39	38.00±1.79	38.33±0.82	37.66±1.99
15 min.	38.58±1.16	37.83±1.72	39.57±1.31	37.58±2.06
30 min.		38.67±1.63	38.08±1.77	38.17±1.57
45 min.			37.67±1.40	38.00±1.22
60 min				37.08±0.58
Lactate (mmol/ L)				
Baseline	2.53±1.07	3.98±3.43	4.70±1.10	4.10±2.76
15 min.	3.16±3.67			
30 min.		2.66±1.22		
45 min.			2.53±0.74	
60 min.				2.38±0.61

Values are given as mean ± SD; n = 6 in each group. MAP: Mean arterial pressure; PaO₂: Pressure of arterial oxygen; PaCO₂: Pressure of arterial carbon dioxide; PET CO₂: End-tidal CO₂.

DISCUSSION

Abdominal compartment syndrome (ACS) was recognized well over a century ago, yet the patho-physiologic implications of IAP were only discovered in the last 20 years. Initially, ACS was considered to manifest only in trauma patients, but we now recognize that it may have significant reverberations throughout the emergency medicine specialty, and that it may also occur in other medical patients.

Three types of ACS have been defined. Primary ACS is essentially associated with organ dysfunction and elevated IAP in the presence of direct injury to the abdominal contents. Secondary ACS typically occurs in patients with severe shock requiring massive resuscitation (e.g., burn patients). Recurrent ACS, which has a very high mortality rate, may occur in patients who have recovered from either primary or secondary ACS.^[2,26]

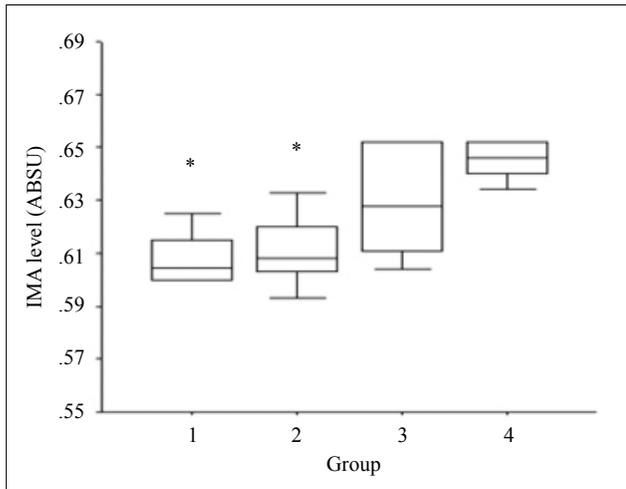


Fig. 1. IMA levels as absorbance unit (ABSU). * $p < 0.05$ for comparison with Group 4.

The clinical diagnosis of increased IAP is difficult and often necessitates invasive procedures such as urinary catheterization, or it is determined by organ failure signs. Delayed or misdiagnosis may result in catastrophic complications and death.^[27] A rapid diagnostic blood test is desirable to assist the clinician in deciding whether to operate on a patient presenting with increased IAP before the signs of organ failure have started in the early periods of ischemia. In this animal study, we studied the value of IMA in sera drawn from the animals after IAP with CO₂ PNM at 15-min intervals up to 1 h, and confirmed our findings by histopathologic examination of the small intestine.

Various studies have hypothesized that IMA is the result of an altered N-terminus of albumin.^[12,14] This truncation of the N-terminus of albumin would decrease the binding of cobalt at this site, resulting in an elevated CABA test. Additionally, abnormally low levels of albumin should result in an elevated CABA test because the amount of cobalt binding is expected to be directly proportional to intact N-terminal albumin levels. In our experimental model, we did not find any statistically significant differences in albumin levels between the study groups. It is well known that many laparoscopic studies have been carried out on rats, and increased IAP may cause changes in some physiologic parameters.^[28] In order to exclude this confounding factor, a rabbit model was used in which we could control these physiologic parameters much more efficiently. During our experiments, we did not see significant changes in vital signs between the study groups. This shows that our study model does not alter vital functions significantly enough to confound the experimental results. We concluded that IMA levels in our experimental model showed the ischemia promptly after 1 h of IAP, which was confirmed by the histopathologic

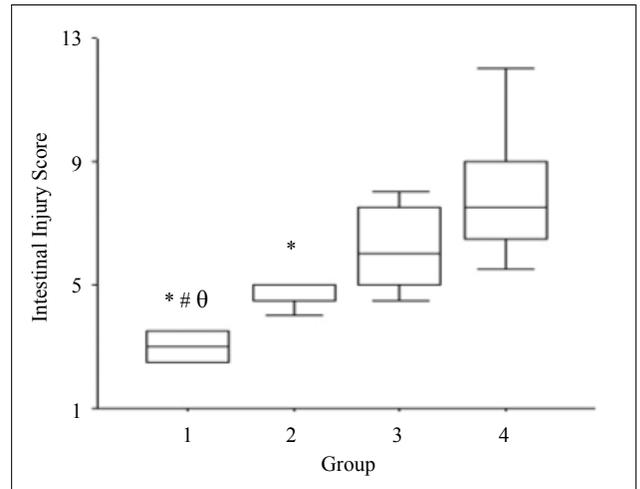


Fig. 2. Intestinal injury score. * $p < 0.05$ for comparison with Group 4; # $p < 0.05$ for comparison with Group 3; θ $p < 0.05$ for comparison with Group 2.

changes of the intestinal tissue samples and was not affected by the albumin levels between the groups.

Laboratory signs, such as increased white blood cell count or serum lactate concentration, are non-specific. C-reactive protein levels, on the other hand, are insensitive in the early stages of IAP and non-specific at later stages.^[29] The signs of organ failure due to ACS are the late findings of IAP. Therefore, research in this field should be directed at defining a simple laboratory test for diagnosis of ACS before the onset of irreversible intestinal ischemia. Although several diagnostic tests to date have been proposed for this purpose, no sensitive and specific marker has yet been discovered. An increasing number of studies have shown that IMA levels rise in a number of acute ischemic conditions, such as cerebral infarct, myocardial ischemia, pulmonary infarct, and acute mesenteric ischemia, for which reason it can be used as a diagnostic marker.^[17,21,22] Mortality and morbidity in ACS are high because of the difficulty of making an early diagnosis.^[30] Experimental work has demonstrated that structural alterations in the albumin begin within 10 min of the onset of superior mesenteric occlusion.^[31] In our experiment, we showed that increased IMA levels after 1 h of IAP correlated with the histopathologic changes in intestinal samples before the signs of organ failure had started.

Apart from invasive tonometric measurements, there are no reliable radiologic or biochemical tests in clinical practice to assist in the diagnosis of ACS or IAP. Our findings indicate that IMA may have a place in the early diagnosis of ACS or IAP in emergency departments or intensive care units. However, large prospective human studies will be needed to determine the place of IMA in the management of this difficult-to-diagnose condition.

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