ORIGINAL ARTICLE

Evaluation of effects of ischaemia on the albumin cobalt binding (ACB) assay in patients exposed to trauma

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Background: In the emergency department (ED), the diagnosis of acute myocardial ischaemia is very difficult because of the absence of a rapid, reliable diagnostic test. The albumin cobalt binding (ACB) assay is a good candidate as a marker for detection of myocardial ischaemia, as it is an easy and rapid test. To date, however, the way in which alterations in metal binding sites of human serum albumin depend on ischaemic events has not been reported in detail.

Methods: We studied 92 patients admitted to the ED within 1 hour after exposure to trauma. Trauma patients divided into two groups according to their Injury Severity Score (ISS): group 1 comprised mildly injured patients who had ISS trauma score <15 ($n=60$), and group 2 comprised moderately injured patients with ISS trauma score >15 ($n=32$). The blood specimens of 30 healthy volunteers were studied as a control group.

Results: Group 2 showed significantly increased ACB levels (0.63 (0.18) absorbance units (ABSU)) compared with group 1 (0.54 (0.14) ABSU) ($p<0.05$) and controls (0.39 (0.05) ABSU) ($p<0.01$). Group 1 showed significantly enhanced ACB values compared with controls (0.54 (0.14) v 0.39 (0.05) ABSU) ($p<0.01$).

Conclusion: Consequently, trauma enhances ACB levels, which may affect the diagnostic performance of the ACB assay, and this effect can limit the ability of the assay for detection of myocardial ischaemia in patients exposed to trauma.

Human serum albumin (HSA) consists of 585 amino acids with nine loops connected with disulphide bonds between cysteine residues.¹ The HSA amino terminal region includes an amino acid sequence, N-Asp-Ala-His-Lys, which has been reported to be a specific binding site for transition metals such as cobalt (II), copper (II), and nickel (II).² This N terminal is the most susceptible region for degradation compared with other regions of albumin.³

In acute coronary syndromes (unstable angina or very early myocardial infarction), after the evolution of the ischaemic event, the in vivo cobalt (II) binding capacity of HSA is reduced because of alterations in the amino terminal sequence.⁴ From this observation, a new marker of myocardial ischaemia, the albumin cobalt binding (ACB) assay was developed.⁵ There have been several studies evaluating the diagnostic performance of the ACB assay in risk stratification of patients with chest pain, but the effects of trauma on the ACB assay has not yet been reported.^{6,7}

The main purpose of this preliminary study was to investigate the performance characteristics of the ACB assay in trauma patients admitted to the emergency department (ED) within 1 hour after exposure to trauma.

METHODS

The study protocol was approved by the Karaelmas University ethics committee, and the study was performed in accordance with the guidelines of the Declaration of Helsinki and its current revision. Informed consent was obtained from parents or guardians before patients were enrolled in the study.

Subjects

We studied 92 patients admitted to the ED within 1 hour after exposure to trauma. This was a randomised controlled study. The cases were patients with multisystem trauma, and were classified for vital signs, and injuries to head and neck,

chest, trunk, and extremities and pelvis. Patients had two or more system injuries. Injuries were identified and selected by Injury Severity Score (ISS).⁸ The patients were assessed for coronary artery disease with 12 lead precordial electrocardiography. Exclusion criteria were: previous myocardial infarction, unstable angina, prior coronary intervention, arrhythmias, conduction abnormalities, heart failure, digoxin therapy, inability to perform tests, chronic renal disease, and diabetes mellitus. Subjects who had chest pain at the time of the study were also excluded. Blood samples were collected at the time the patient was first admitted to the ED. The trauma patients were divided into two groups according to ISS: group 1 were mildly injured patients with ISS <15 ($n=60$), while group 2 were moderately injured patients with ISS >15 ($n=32$). Group 1 comprised 35 men (mean (SD) age 43.4 (18.4) years) and 25 women (44.0 (18.7) years), and group 2 comprised 24 men (45.3 (15) years) and 8 women (34 (19.7) years).

Serum specimens were also collected from the control group, which comprised 30 healthy individuals (15 men and 15 women, aged 30.0 (10.0) years) with no evidence of myocardial ischaemia, no personal or family history of cardiovascular disease, and no known cardiovascular risk factors.

Laboratory tests

Serum creatine phosphokinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) were measured by the enzymatic method, and albumin concentrations were detected with by colorimetry on an automatic analyser (Roche Integra 800 autoanalyser). Serum myoglobin

Abbreviations: ABSU, absorbance units; AST, aspartate aminotransferase; CK, creatine phosphokinase; cTnI, cardiac troponin I; ED, emergency department; HSA, human serum albumin; ISS, Injury Severity Score; LDH, lactate dehydrogenase

levels were measured by electrochemiluminescent immunoassay (Roche Elecsys 2010 analyser). Serum cardiac troponin I (cTnI) levels were measured by chemiluminescent immunoassay (Beckman Coulter Access II). cTnI concentrations >0.04 were considered positive. ACB levels were measured by spectrophotometry (Shimadzu UV-1601) using Bar-Or's method.³

Statistical analysis

Data were reported as mean (standard deviation (SD)). Differences between the patient and control groups were compared using Student's *t* test. For correlation analysis, Pearson's correlation was used. All statistical analyses were performed with SPSS software (version 11.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Group 2 showed significantly enhanced mean (SD) ACB values (0.63 (0.18) absorbance units (ABSU)) compared with group 1 (0.54 (0.14) ABSU) ($p = 0.045$) and the control group (0.39 (0.05) ABSU) ($p < 0.01$). Group 1 showed significantly increased ACB levels (0.54 (0.14) ABSU) compared with controls (0.39 (0.05) ABSU) ($p < 0.01$) (fig 1). None of the patients had cTnI levels >0.04 and there was no significant differences between group 1, group 2, and controls (0.014 (0.006), 0.015 (0.007), and 0.012 (0.005), respectively). The biochemical markers AST, CK, LDH, and myoglobin showed significant correlation between trauma severity ($r = 0.895$, $p < 0.01$; $r = 0.923$, $p < 0.01$; $r = 0.852$, $p < 0.01$; $r = 0.909$, $p < 0.01$, respectively) but there was no relationship between ACB and severity of trauma ($r = 0.137$, $p = 0.205$). In patients, albumin concentrations were within the reference range (3.4 to 4.8 g/dL): mean (SD) albumin, 4.07 (0.51) g/dL; minimum, 3.4 g/dL; maximum, 4.8 g/dL (table 1).

DISCUSSION

Preliminary studies on acute coronary syndromes have been shown that the ACB test is a promising candidate for early prediction of myocardial ischaemia.^{6,7} The assay has both a high sensitivity and a negative predictive value for the identification of acute coronary syndromes, in combination with electrocardiography and troponin testing.⁶ Bhagavhan *et al* found that at a cut off value of 0.50 ABSU, the sensitivity and specificity were 88% and 94%, respectively, for identifying individuals with myocardial ischaemia from non-ischaemic individuals.⁹

In our study, there was a significant increase in AST, CK, LDH, and myoglobin levels in trauma patients, and these parameters showed significant correlation with the severity of trauma. Thus, it seems that the use of these biochemical markers in the diagnosis of myocardial ischaemia is limited in trauma because exposure to trauma is responsible for large increases in myoglobin and enzyme release from the skeletal

muscle. In contrast to these markers, there was no difference for cTnI between the groups. From these results, we suggest that high ACB values limit the ability of the test for detection of myocardial ischaemia in trauma patients. However, enhanced serum ACB levels had no relationship to the severity of trauma. This novel finding shows that increased ACB values are also related to trauma, but the association between ACB and trauma was independent of severity and was seen in both mildly and moderately injured subjects.

Myocardial ischaemia induces anaerobic metabolism, which results in increased lactic acid and reduced pH. Acidosis has been reported to affect metal binding to HSA,¹⁰ but there have been few reports regarding ischaemic effects on albumin-metal binding sites.⁴ Bar-Or *et al* showed transient increased ACB levels during elective percutaneous transluminal angioplasty, which returned to baseline values within 6 hours.¹¹ Although the exact mechanism that causes enhanced ACB values during ischaemia is not known, it seems to be reversible. In accordance with the previous study, our trauma patients displayed high ACB values in the short term after trauma, which could be attributed to transient ischaemia caused by injury.

Trauma patients are known to have hypoalbuminaemia even in the first hour after the injury,¹² and low albumin concentration will certainly lower cobalt uptake and therefore increase the ACB levels. We observed serum albumin values for all patients within the reference range, thus we can say that high ACB levels in trauma patients may not be due to low albumin concentrations.

In conclusion, serum ACB levels are elevated in trauma. Based on these data, we conclude that the ACB assay cannot be used reliably to indicate myocardial ischaemia in injured patients.

Table 1 Comparison of mean (SD) serum parameters of group 1 (mildly injured patients that has ISS trauma score below 15) and group 2 (moderate injured patients that has ISS trauma score above 15) with those of the controls

Parameter	Group 1 (n=60)	Group 2 (n=32)	Control (n=30)
ACB (ABSU)	0.54 (0.14)*	0.63 (0.18)†‡	0.39 (0.05)
cTnI (ng/ml)	0.014 (0.006)	0.015 (0.007)	0.012 (0.005)
Myoglobin (ng/ml)	121.8 (107.5)*	653.3 (626.7)†‡§	21.7 (15.6)
CK (U/l)	293.5 (215.4)*	373.7 (249.2)†§	106 (22.6)
LDH (U/l)	454.9 (285.1)*	850.5 (578.0)†§	135.5 (14.8)
AST (U/l)	43.5 (46.7)*	75.8 (72.4)†§	22.1 (15.2)

Data are mean (SD). Significant difference between: *control group and group I ($p < 0.01$); † control group and group II ($p < 0.01$); ‡ groups I and II ($p < 0.05$); § groups I and II ($p < 0.01$)

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REFERENCES

- 1 **Meloun B**, Moravek L, Kostka V. Complete amino acid sequence of human serum albumin. *FEBS Lett* 1975;**58**:134–7.
- 2 **Sadler PJ**, Tucker A, Viles JH. Involvement of a lysine residue in the N-terminal Ni^{2+} and Cu^{2+} binding site of serum albumins. Comparison with Co^{2+} , Cd^{2+} , Al^{3+} . *Eur J Biochem* 1994;**220**:193–200.
- 3 **Chan B**, Dodsworth N, Woodrow J, *et al*. Site-specific N-terminal auto degradation of human serum albumin. *Eur J Biochem* 1995;**227**:524–28.
- 4 **Bar-Or D**, Lau E, Rao N, *et al*. Reduction in the cobalt binding capacity of human albumin with myocardial ischemia. *Ann Emerg Med* 1999;**4**:34–56.
- 5 **Bar-Or D**, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia a preliminary report. *J Emerg Med* 2000;**19**:311–15.
- 6 **Wu AH**, Morris DL, Fletcher DR, *et al*. Analysis of the albumin cobalt binding (ACB) test as an adjunct to cardiac troponin I for the early detection of acute myocardial infarction. *Cardiovasc Toxicol* 2001;**1**:147–51.
- 7 **Christenson RH**, Duh SH, Sanhai WR, *et al*. Characteristics of an albumin cobalt binding test for assessment of acute coronary syndrome patients: a multicenter study. *Clin Chem* 2001;**47**:464–70.
- 8 **Baker SP**. The Injury Severity Score: a method for describing patients with multiple injuries and evaluating emergency care. *J Trauma* 1974;**14**:187–96.
- 9 **Bhagavan NV**, Lai EM, Rios PA, *et al*. Evaluation of human serum albumin cobalt binding assay for the assessment of myocardial ischemia and myocardial infarction. *Clin Chem* 2003;**49**:581–5.
- 10 **Cobbe SM**, Poole Wilson PA. The time onset and severity of acidosis in myocardial ischemia. *J Mol Cell Cardiol* 1980;**12**:745–60.
- 11 **Bar-Or D**, Winkler JV, Vanbenthuyzen K, *et al*. Reduced albumin-cobalt binding with transient myocardial ischemia after elective percutaneous transluminal coronary angioplasty: a preliminary comparison to creatine kinase-MB, myoglobin, and troponin I. *Am Heart J* 2001;**141**:985–91.
- 12 **Richard L**, Yukl, David Bar-Or, Lisbeth Harris, *et al*. Low albumin level in the emergency department: a potential independent predictor of delayed mortality in blunt trauma. *J Emerg Med* 2003;**25**:1–6.