

Can ischemia-modified albumin be used to differentiate between generalized seizures and pseudoseizures?

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Background/aim: In recent years ischemia-modified albumin (IMA) has been suggested as a marker that can be used in differentiating nonconvulsive conditions from epilepsy. The purpose of this study was to investigate changes in IMA levels caused by generalized clonic tonic (GTC) seizures.

Materials and methods: A total of 114 children presenting to the Karadeniz Technical Pediatric Emergency Polyclinic with GTC seizures were included in the study. Sixteen cases meeting the inclusion criteria were included in the study and sixteen healthy children were enrolled as the control group. The patients' IMA, albumin, and IMA/albumin values at hours 0 and 1 following the episode were compared with control group values.

Results: IMA levels in the patient group were significantly higher at hour 1 compared to hour 0, and were also significantly higher than those of the control group levels at hour 1. In addition, the patient group IMA/albumin index value at hour 1 was significantly higher than the baseline value. IMA levels increased significantly with length of seizure.

Conclusion: Although there were no markers of hypoxia in patients undergoing GTC seizures in this study, hypoxia was observed to develop, and this caused serum IMA levels to rise in line with seizure duration.

Key words: Ischemia-modified albumin, generalized clonic tonic, albumin, IMA/albumin

1. Introduction

The metal-binding capacity of albumin for transition metals, such as copper, nickel, and cobalt, is reduced during acute ischemic conditions. This results in a metabolic variant of the protein ischemia-modified albumin (IMA) (1). The observation that myocardial ischemia produced a lower metal-binding capacity for cobalt to albumin led to the development of the albumin-cobalt binding (ACB) test, which was recently approved by the FDA. The ACB test is a quantitative assay that measures IMA in human serum (2). Experimental and clinical studies have shown an increase in IMA levels in blood in various organ ischemias or injuries, particularly myocardial ischemia, and systemic ischemias (2–9). One study of patients with PCI reported that IMA is not only a marker of the occurrence of an ischemic event, but also an indicator of the severity of ischemia (10).

Seizures, particularly generalized tonic clonic (GTC) seizures, give rise to a number of physiological consequences. Prolonged seizures result in deterioration of homeostatic mechanisms (11). Ictal hypoxemia has been studied extensively in children and adults (12,13). In a study of 225 seizures and 49 epileptic children, Moseley et al. (13) reported ictal hypoxemia in 48.9% of children and in 26.8% of seizures. In that study, ictal hypoxemia was also significantly more likely to occur during generalized rather than nongeneralized seizures (43.9% vs. 18.9%) and when tapering antiepileptic drugs (75% vs. 35.5%) (13). Secondary physiological changes (including hypotension, hypoxia, hypoglycemia, and hyperthermia) occurring as the length of epileptic seizures increases contribute to injury in both the brain and other organs (14).

Ictal hypoxemia during seizure is a well-known condition. The ability to use biochemical markers to

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determine this condition may be beneficial. The purpose of this study was to determine whether IMA, regarded as a good biochemical marker of cerebral or systemic ischemia/hypoxemia, alters in cases with no markers of hypoxia following seizures (no acidosis, oxygen saturation >90%), and to test the association with length of seizure. For that purpose we compared serum blood IMA levels of patients undergoing GTC seizures at hours 0 and 1 with IMA levels in an age- and sex-matched control group at hours 0 and 1.

2. Materials and methods

This study was conducted at the Karadeniz Technical University Hospital Department of Pediatric Neurology, Turkey, with the approval of the institutional ethics committees and in conformity with the Helsinki Declaration, ICH/GCP, and local regulations.

A total of 114 children presenting to the Karadeniz Technical Pediatric Emergency Polyclinic with GTC seizures and undergoing GTC seizures while under observation at the EEG monitoring unit were considered for the study. Patients with seizure duration of 1–5 min, with postseizure oxygen saturation of >90% and no acidosis (pH of >7.35), with no chronic or degenerative disease (diseases of the cardiovascular or respiratory systems, diabetes, etc.), with negative acute phase proteins and fever, and with no other epileptic episode in the previous 24 h were selected. Sixteen patients meeting the inclusion criteria (9 males, 7 females) were included in the study. Blood specimens were collected at hours 0 and 1. Sixteen (9 males, 7 females) age- and sex-matched children with no disease being monitored at the healthy child clinic were enrolled as the control group.

Patients' IMA, albumin, and IMA/albumin values at hours 0 and 1 were compared with control group values.

2.1. IMA measurement

Blood samples were placed into plain tubes containing separation gels. The samples were allowed to clot for 30 min and were then centrifuged before separating the serum. The samples were then immediately frozen and stored at -80°C for IMA assays.

Reduced cobalt-to-albumin binding capacity (IMA level) was analyzed using the rapid colorimetric method described by Bar-Or et al. (15). Two hundred microliters of patient serum was placed into glass tubes, and 50 μL of 0.1% cobalt chloride (Sigma, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) in H_2O was added. After gentle shaking, the solution was left for 10 min in order to ensure sufficient cobalt-albumin binding. Fifty microliters of dithiothreitol (DTT) (Sigma, 1.5 mg/ mL H_2O) was added as a colorizing agent. The reaction was quenched 2 min later with the addition of 1.0 mL of 0.9% NaCl. A colorimetric control was prepared for preoperative and postoperative serum samples. For the colorimetric

control samples, 50 μL of distilled water was substituted for 50 μL of 1.5 mg/ mL DTT. Specimen absorbencies were analyzed at 470 nm on a spectrophotometer (Shimadzu UV1601, Australia). The color of the DTT-containing specimens was compared with that of the colorimetric control tubes. The results were reported as absorbance units (ABSU).

2.2. Statistical analysis

Data analysis was performed using SPSS 11.5. Descriptive statistics are shown as mean \pm SD. Normality of distribution (normality test - Kolmogorov-Smirnov test) was tested in the measured values. Since the data complied with normal distribution, parametric tests were used in analyses. Treatment and control groups were compared in terms of IMA, albumin, and IMA/albumin. Student's t-test was used to compare IMA, albumin, and IMA/albumin (at hour 0 for the control group and at hour 0 postseizure in the patient group; and at hour 1 in the control group and hour 1 postseizure in the patient group). The paired t-test and Student's t-test were used to compare two values within the patient group and with the controls (hour 0 and hour 1 values), respectively. The significance of linear correlation between changes in IMA and length of seizure (in seconds) was examined using Spearman's correlation test. Significance was set at $P < 0.05$.

3. Results

The mean age of the 16 patients in the patient group was 9.87 ± 2.33 years, and mean age was 9.69 ± 2.33 years in the 16 control group subjects. Both groups consisted of 9 boys and 7 girls. The groups' IMA and albumin levels and IMA/albumin index values are shown in the Table.

Control group IMA levels at hour 1 were lower than at hour 0 ($P < 0.05$). No change was observed in control group albumin levels between hours 0 and 1, and no difference was observed between IMA/albumin index values.

In the patient group, IMA levels at hour 1 increased significantly compared to those at hour 0, and they were also significantly higher than the control group values at hour 1 ($P < 0.05$ and $P < 0.001$, respectively). Patient group albumin levels at hours 0 and 1 were significantly lower than the corresponding levels in the control group. Albumin levels at hour 1 in the patient group were significantly lower than the values at hour 0 ($P < 0.05$). IMA/albumin index values in the patient group at hours 0 and 1 were significantly higher than the corresponding values in the control group ($P < 0.05$ and $P < 0.001$, respectively). In addition, IMA/albumin index values in the patient group at hour 1 were significantly higher than the baseline value ($P < 0.01$).

A comparison of IMA levels at hours 0 and 1 in the control and patient groups is shown in Figure 1. Patients' lengths of seizures were recorded (in seconds). A

Table. IMA, albumin, and IMA/albumin values in the patient and control groups.

	Controls (n = 16)		Patients (n = 16)	
	Hour 0	Hour 1	Hour 0	Hour 1
IMA	0.552 ± 0.030	0.533 ± 0.043	0.586 ± 0.082	0.652 ± 0.082
Albumin	4.56 ± 0.22	4.54 ± 0.21	4.19 ± 0.21	4.04 ± 0.25
IMA/albumin	0.121 ± 0.011	0.118 ± 0.013	0.140 ± 0.026	0.158 ± 0.023

IMA: Ischemia modified albumin.

Values are expressed as mean ± SD; the paired samples t-test was used for intragroup analysis and Student's t-test for intergroup analysis.

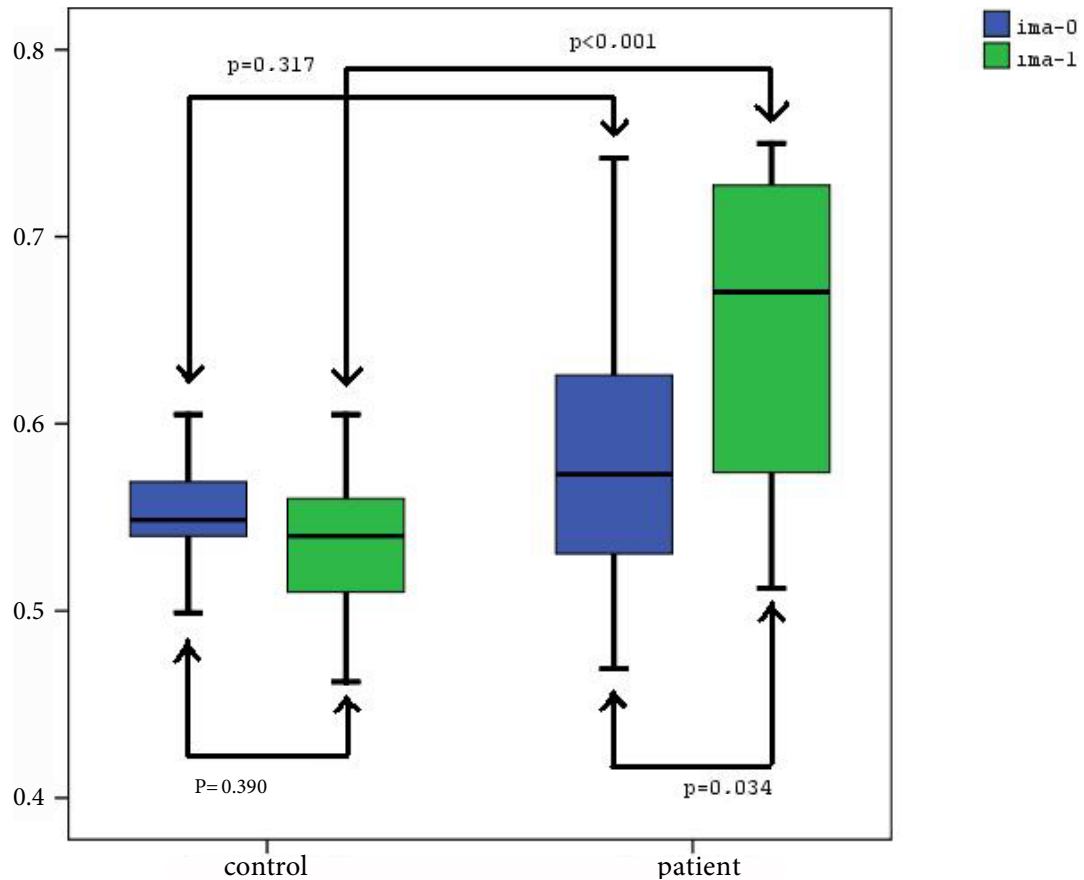


Figure 1. Ischemia-modified albumin level changes in generalized tonic clonic seizures.

significant correlation was determined between duration of seizure and IMA values, with IMA values increasing with length of seizure (Figure 2).

4. Discussion

IMA increases in myocardial ischemia, cancers, infections, end-stage renal disease, liver disease, and cerebral ischemia (2–10,16). This study shows that serum IMA levels increase even in the absence of hypoxia markers in

patients undergoing GTC seizures and that serum IMA values are directly proportional to length of seizure.

The number of studies investigating IMA levels in conditions involving the central nervous system is limited, and the findings are inconsistent. Turedi et al. (17) noted the presence of elevated IMA levels in carbon monoxide-poisoned patients and suggested that IMA is sensitive to hypoxia. Abboud et al. (18) examined 118 patients (84 brain infarctions, 18 brain hemorrhages, and 16 transient

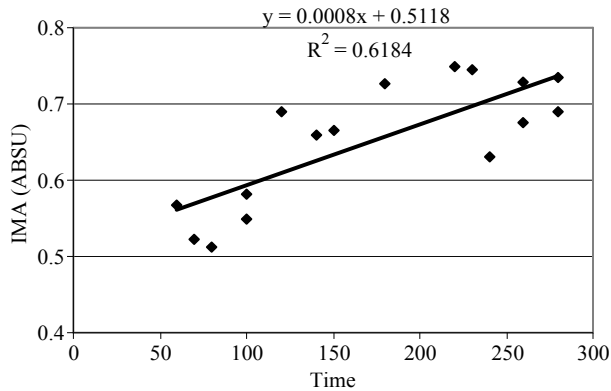


Figure 2. Correlation between IMA levels and length of seizure (in seconds). A significant positive correlation was determined between length of seizure and IMA levels ($r = 0.79$; $P < 0.001$).

ischemic attacks lasting less than 1 h or epileptic seizures) presenting within 3 h of acute neurological deficit in order to investigate whether or not stroke affects blood IMA levels. That study measured IMA in blood specimens collected from all patients at baseline and from patients with stroke collected at hours 6, 12, and 24. They reported that IMA levels rose in the first 24 h in patients with cerebral infarction but remained unchanged in patients with cerebral hemorrhages, and they concluded that IMA levels might be a biomarker for the early diagnosis of acute stroke. Ahn et al. (19) compared the albumin-adjusted IMA index and IMA values in patients with ischemic stroke. They showed that this IMA index was a more sensitive diagnostic biomarker for stroke than conventional IMA values but that its value in predicting progression was questionable. Haklıgör et al. (20) suggested that the ACB assay reflects albumin concentrations rather than IMA levels, and that albumin concentration is a primary predictor of IMA in serum. They observed a strong negative relationship between albumin concentrations and IMA, and they reported that IMA values were directly determined by albumin concentrations at all albumin levels. Since measurement of IMA values using the ACB technique relies on measurement of cobalt that does not bind to albumin, IMA level results will be affected by albumin concentrations in serum. It is well known that ACB test results should be interpreted with caution when serum albumin concentrations are <20 g/L or >55 g/L, or in the presence of increased lactate or ammonia concentrations (2). The IMA index may provide more accurate results by compensating for this effect. The albumin levels in our study were at appropriate intervals for interpretation. All patients' blood gas values were normal. IMA index values were also used in comparison of the patient and control groups in order to neutralize the effect of albumin values. Our study shows that IMA levels and IMA index levels

rose in the 1st hour in patients undergoing GTC seizures and were significantly higher than control group values. It also shows a significant positive correlation between length of GTC seizure and IMA levels.

There are also study findings suggesting limited clinical potential of IMA as an ischemic marker. Kim et al. (21) concluded that in clinical practice the use of serum IMA level does not seem beneficial, neither for the triage of patients with acute chest pain nor for risk stratification of patients with ischemic chest pain. Herisson et al. (22) concluded that IMA does not appear to be relevant in acute stroke management. It has also been reported although IMA is not highly specific as a marker of ischemia, it is quite sensitive when negative (23).

The main limitations of this study are the low case numbers enrolled and the difficulty in interpreting the findings. In conclusion, this study shows that certain levels of hypoxia develop in patients undergoing GTC seizures even in the absence of hypoxia markers (such as acidosis and low oxygen saturation), and that this gives rise to an increase in IMA levels proportional to length of seizure. Investigation of postseizure serum IMA levels can therefore provide information concerning hypoxia during seizure or length of seizure.

In addition, our findings may also show that serum IMA levels can particularly be used as a biochemical marker in differentiating nonepileptic and epileptic seizures. The fact that history of seizure in patients presenting to the emergency department is generally obtained from families sometimes makes it difficult to differentiate between seizure and pseudoseizure. Families are unable to provide sufficient anamnesis in such circumstances due to the fear and panic caused by the event. Even if we make use of EEG in such conditions, we know that interictal EEG recordings may be normal in many patients. We therefore think that other markers are needed to differentiate between seizure and pseudoseizure.

Patients' lengths of seizures were recorded (in seconds). A significant correlation was determined between duration of seizure and IMA values, with IMA values increasing with length of seizure (Figure 2). In this study, in addition to an increase in IMA values in generalized seizures, we also determined a linear correlation between length of seizure and that IMA values increased with duration of seizure. We think that in addition to contributing to differentiating pseudoseizures from seizures, this will also provide information concerning the duration of seizure in patients brought to our hospital due to seizures. Due to the low number of patients meeting the study criteria, we were unable to compare IMA levels in longer and shorter seizures with different groups and durations of seizure. Further studies involving larger patient numbers will make such comparison possible.

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