

## Serum Adenylate Kinase Activity in the Early Phase of Acute Myocardial Infarction

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### ABSTRACT

Elevated adenylate kinase activities in serum and urine have been studied among patients suffering from myocardial infarction. 71% of the patients had clearly increased activities in serum already on admission to the hospital, i.e. about 12 hours after onset of symptoms and patients was 45%. A maximum peak value of the activity in serum was seen at 6 hours after admission to the hospital, i.e. about 12 hrs after onset of symptoms and the elevated enzyme activity value persisted for at least 12 hours after admission. The patients with the elevated adenylate kinase activity in serum were arbitrarily divided into three groups as regards activity. It was shown that the difference in activity of ASAT as well as LD of the high and low activity group was significant ( $p < 0.01$ ). No such difference was established between these groups for ALAT, usually not being considered as a reliable test enzyme for myocardial infarction.

### INTRODUCTION

The importance of determination serum enzyme levels in patients suspected of having an acute myocardial infarction has been well documented. The fact that the WHO criteria for acute myocardial infarction include enzyme activity profiles of ASAT, ALAT as well as the LD isoenzymes is an expression for that.

Since difficulties have arisen in finding an enzyme specific enough for the myocardium, several different enzymes appearing in the serum have been used as a diagnostic aid for establishing myocardial infarction (1, 10). It has been claimed recently that the isoenzymes of creatine kinase represent a higher degree of specificity (8). A similar high degree of specificity has been attributed to arginase (7) and glycogen phosphorylase (4) in the early differential diagnosis of myocardial infarction.

Adenylate kinase catalyses a dismutation reac-

tion between two molecules of adenosine diphosphate (ADP) giving rise to one molecule of adenosine triphosphate (ATP) and one adenosine monophosphate (AMP). The enzyme occurs in heart and skeletal muscle in concentrations higher than in other organs (6). It is partly free in the cytoplasm and partly bound to subcellular organelles especially to the mitochondrion. It also appears in human erythrocytes. The molecular weight is 21,000. Therefore it is reasonable to assume that the enzyme penetrates the plasma membrane relatively easy even after a slight damage to the cell and appears in the blood plasma. Due to similar reasons it should easily enter into the urine. Only a few reports have so far dealt with adenylate kinase in serum in connection with myocardial infarction (2, 5, 9).

We report in the present communication on the early rise of adenylate kinase in serum as well as in urine after myocardial infarction.

### MATERIAL AND METHODS

26 consecutive patients, 19 men and 7 women (age range 33–80 years) with myocardial infarction were included in the study. The diagnosis of myocardial infarction was based upon the following three criteria.

(a) *Clinical history*—typical clinical history with acute central chestpain of at least 15 minutes duration with or without frank pulmonary oedema or shock.

(b) *Positive electrocardiographic findings*—the appearance of a Q-wave and/or S-T elevations followed by subsequent T-inversions in a localized area.

(c) *Positive serum enzyme pattern*—typical enzyme pattern with an increase of ASAT with lower ALAT and/or increase of LD in a characteristic time course.

At least two of these three criteria should be fulfilled. 21 patients had all three criteria. The study was carried out in the Coronary Care Unit of the Medical Department. 25 patients were admitted to the hospital within 6 hours after the onset of symptoms. In one case, there

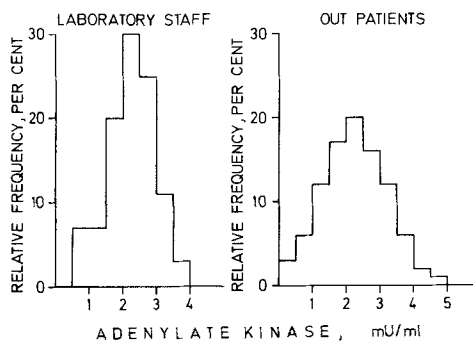


Fig. 1. Distribution of serum adenylate kinase activity of two categories of normals.

was no sure information about the time which had elapsed between the start of the symptoms and admission. 19 had first time infarction, while the others had recurrences. One patient had in addition congestive heart failure due to an earlier infarction. No one had signs of myopathia, nephropathia or liver disease.

Blood samples and urine were obtained repeatedly from the patients according to the following scheme. Immediately on arrival the first blood sample was drawn and a urine portion voided. After that, blood was drawn every 6 hours up to 24 hours after arrival. Urine was collected during the first 24 hours, and another portion between 24–48. According to the usual routine, samples for ASAT, ALAT and LD were drawn once daily for 3 days. The patients were treated in accordance with the standard schedule for myocardial infarction. Intramuscular injections were avoided for this study with two exceptions.

#### Controls

27 healthy members of the laboratory staff (aged 25–60) served as one control group and 83 out-patients (age range 18–91) on health control as another. From the controls one single blood sample was obtained and handled in the same way as for patients. Urine was collected between 11 p.m. and 7 a.m.

#### Laboratory methods

All chemicals were of analytical grade. ATP, ADP and AMP were purchased from Sigma Chemical Company, St Louis, Mo., USA. NADH, phosphoenolpyruvate (tricyclohexylammoniumsalt) and the enzymes were obtained from Boehringer & Soehne, GmbH, Mannheim, Germany.

**Analytical methods:** The blood samples to be analysed were immediately brought to the laboratory and centrifuged twice in order to avoid any further blood cell contamination of the serum. If not immediately analysed the serum was kept frozen at  $-20^{\circ}\text{C}$  until analysis.

Before enzymatic determination any hemoglobin contamination of serum was ruled out by direct measurement at the wave-lengths 420 nm (the Soret band) and 540 nm, respectively.

**Enzyme assay:** The adenylate kinase determination was performed in an assay medium buffered with triethanola-

mine-hydrochloric acid buffer, pH 7.6, 0.05 M with respect to total triethanolamine. The activity of the enzyme was estimated by coupling ADP formation with pyruvate kinase, phosphoenolpyruvate, lactic acid dehydrogenase and NADH. Correction was made for the slow reaction obtained in the absence of AMP. The adenylate kinase was assayed by means of enzymatic reactions terminating in the stoichiometric oxidation of the pyridine nucleotide, with conditions adjusted so that the adenylate kinase to be measured was rate-limiting in the overall reaction. The reduced pyridine nucleotide was measured at 340 nm in a Zeiss PM QII spectrophotometer. The assay medium was in a 1 cm quartz cuvet at  $25^{\circ}\text{C}$ . A molar extinction coefficient of  $6.22 \times 10^3/\text{cm}$  for NADH was assumed (3). Enzyme activities are expressed in units per ml of serum or urine. One unit is defined as that amount of enzyme, which oxidizes one  $\mu\text{mol}$  of NADH per min at  $25^{\circ}\text{C}$ .

## RESULTS

The mean values of adenylate kinase in serum from the two categories of normals did not differ significantly from each other. Thus, a mean value of 2.3 mU/ml was measured for the laboratory staff. The corresponding figure for the out-patients was 2.2 mU/ml (Fig. 1). The upper limit of normal values was considered to be 4.25 mU/ml (Mean + 2 S.D.). A minimal background activity of adenylate kinase was sometimes registered and therefore urine was considered negative when this activity did not exceed 1.5 mU/ml.

15 patients out of the 21 fulfilling all three criteria for myocardial infarction had elevated levels of adenylate kinase already on arrival. This elevation persisted for at least 12 hours except in one case, which was the oldest patient, a man 80 years old. Fig. 2 shows the mean adenylate kinase value at different times after admission. It is seen that a

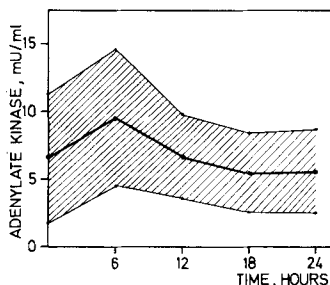


Fig. 2. Mean adenylate kinase activity  $\pm 1$  S.D. in serum of patients with acute myocardial infarction on admission and after certain time intervals.

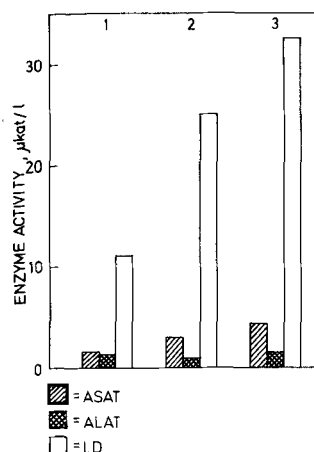


Fig. 3. Comparison with other myocardial enzymes. The patients have been grouped according to adenylate kinase activity into low (1), medium (2) and high (3) activity groups, respectively.

maximum value of 9.5 mU/ml is obtained at 6 hours, i.e. within 12 hours after the onset of symptoms. The range within  $\pm 1$  S.D. was 4.5–14.5 mU/ml. However, the peak is not very pronounced. The highest value observed, 23.0 mU/ml, was found in one patient already on admission. Furthermore, it is clear from the figure, that increased adenylate kinase levels persisted even after 24 hours.

In order to compare the amount of adenylate kinase in serum from these patients with that of the transferases and LD the material was divided arbitrarily into three groups as regards adenylate kinase activity. Thus the high activity group comprised activities over and above 10.0 mU/ml ( $n=9$ ) the medium contained samples within the activity range 6.30–9.95 mU/ml ( $n=9$ ) and the low range 4.30–6.25 mU/ml ( $n=7$ ).

Within each group the mean peak values of ASAT, ALAT and LD were calculated. It is obvious from Fig. 3 that a correlation exists between high adenylate kinase and high activities of ASAT and LD. No such correlation was displayed for ALAT. The difference in activity in ASAT and LD was statistically significant ( $p<0.01$ ) between the high and low adenylate kinase activity groups. No such difference could be established between these groups for ALAT.

The adenylate kinase activity in urine is shown in Table I. It is seen that 11 patients had clearly elevated levels, 3 were borderline and 11 were

normal. Among the patients with positive urine as regards adenylate kinase, 9 out of 11 had elevated levels already in the first urine portion.

## DISCUSSION

According to our results almost every patient with acute myocardial infarction had elevated adenylate kinase activity in serum. This increase in adenylate kinase activity appeared early in the course of the disease. Most patients had increased activity already on admission, i.e. within 6 hours after the onset of symptoms. Only one out of 26 patients failed to show that pattern. It is reasonable to believe that in this case the admission to hospital had been delayed.

The pathological elevation of adenylate kinase in serum did not show any sharp peak, instead the curve had a rather flat course. This could be expected according to the low molecular weight of the enzyme (21,000) which means high clearance in the kidney. 9 out of 20 patients also had pathological adenylate kinase activity in urine already on admission which support this statement.

Among these patients with myocardial infarction and increased adenylate kinase activity there was a positive correlation between the adenylate kinase level and the levels of other infarction enzymes. When interpreting such a correlation it must however be born in mind that the different enzymes appear in time on different stages of the disease. The adenylate kinase is not heart-specific, as mentioned before. In these patients, however, there were no muscular trauma, signs of liver disease and intramuscular injections were avoided as far as possible. It must be borne in mind that the

Table I. Urinary adenylate kinase activity among patients with myocardial infarction

Activity in urine, degree	n	Positive in first sample n	Positive in second sample n
Strongly positive	8	8	0
Positive	2	1	1
Borderline	2	1	1
Negative	8		
Missing	6		
Total	26	10	2

erythrocytes contain adenylate kinase and therefore hemolysis can influence the results. Therefore, since the enzyme can easily pass a disarranged red cell membrane and furthermore be eluted from the surface of the plasma membrane it is important that the blood samples to be analysed are taken under completely standardized conditions as regards needle thickness, the centrifugation procedure and the complete avoidance of hemolysis.

Provided a complete clearance of adenylate kinase occurs in the kidney, one would expect a positive finding in the urine of the majority of the patients with myocardial infarction. However, we found positivity in 45% of these patients. One explanation to that might be, that the enzyme is adsorbed to the renal tubuli cells and never reach the urine. Another reason might be an inactivation of the enzyme in the urine by oxidation of mercapto-groups, for instance, the intactness of which is necessary for the enzyme function.

Adenylate kinase seems to offer interesting prospects in the early diagnosis of myocardial infarction. It appears early in the acute phase and is easy to determine. Furthermore, its presence in certain cases in urine early in the course of the disease gives additional aid in the rapid and simple laboratory investigation for myocardial infarction.

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