CASE REPORTS

A CASE OF CREATINE KINASE ISOMER ANOMALY IN RELATION TO THE DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION

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Creatine kinase (CK) isomers have recently been suggested to be of value in the diagnosis of acute myocardial infarction (AMI). The aim of this report was to determine whether measurement of CK isomers, especially the MM isomer and the cardiac-specific marker MB isomer, is practical in the diagnosis of AMI. A 64-year-old female with consistently high values for total CK showed an electrophoretic pattern which suggested AMI. We ruled out all possible causes of increased CK, and its MB isomer, including IgGβ, beta-LP (lipoprotein) and mitochondrial complexes as well as myocarditis, muscular disorders, and myoglobinuria. Regardless of the source of the anomaly, the fact remains that CK macroanomaly cases can be an obstacle in the diagnosis of AMI. CK isomers have proven to be accurate markers in AMI and valuable in doubtful cases, such as non-O-AMI. However, in light of this particular case of CK macroanomaly, isomers are not 100% accurate (specific) in the diagnosis of AMI.

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In all of the patients who are admitted to our Emergency Department with symptoms of acute myocardial infarction (AMI), we measure creatine kinase (CK) isomer isoenzymes and lactate dehydrogenase (LDH) isoenzymes, using a rapid electrophoresis analyzer. Our observations have demonstrated that the MB and MM isomers are sensitive indicators for the diagnosis of AMI, and are especially useful in cases which are difficult to diagnose by electrocardiogram (ECG) when no ST-T changes or Q wave patterns are elicited.1−3 While there have been previous reports of macroanomaly regarding isoenzymes of CK and LDH, this report is, to the best of our knowledge, the first regarding isomer-related anomaly.4−7 It is well documented that most anomalous cases are due to binding of IgG immunoglobulin with CK-BB, which usually migrates between CK-MM and CK-MB on electrophoresis.5−12 At other times, CK-MB has been known to co-migrate with CK-MM. Anomalous CK of mitochondrial origin migrates cathodal to CK-MM.13 On agarose gel, the anomalous macro-CK of this patient migrated with an electrophoretic mobility between those of CK-MM and CK-MB, and was closer to the immediate anodal aspect of CK-MM. The macroanomaly pattern of this patient interfered with the interpretation of CK isoenzyme and isomer analysis, leading to a false positive diagnosis of AMI. The difficulty was much more pronounced with the isomers than with the isoenzymes, since

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AMI (acute myocardial infarction)
CPK (creatine phosphokinase)
CPK isoforms (isomers)

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At admission (Feb. 16, '92)

3 Weeks after admission (Mar. 10, '92)

Fig. 1. ECG (a) At admission (negative T wave in the 1 and AVL leads). (b) Three weeks after admission.

5 ml collected (blood)
Centrifuged for 10 mins at 3000 rpm
serum decanted

75 μl of pipetted into each sample cup.
After placing reagent, gel plate into appropriate place the REP unit automatically performs electrophoresis, then incubates and dries the agarose gel.

The isoform gel is visually inspected under U. V light
Finally the densitometer will automatically calculate and print the CK-MB and CK-MM values on the computer screen.

SUMMARY OF CONDITIONS
Gel REP CK isoforms-15 Gel
Sample volume 7.50 μl
Electrophoresis Temp 10 °C
Electrophoresis Time 15:00 min
Current 0 mA
Voltage 1400 volt
Incubation Time 4:30 min
Incubation Temp 45 °C
Drying Time 8:00 min
Drying Temp 54 °C
Scanning Wavelength Fluorescence Mode

Fig. 2. Schematic illustration of the procedure. "5 ml of blood is collected and centrifuged for 10 min at 3000 rpm. Serum is decanted 75 ml of serum is pipetted into each sample cup. After reagent and the gel plate are placed into appropriate places, the REP unit automatically performs electrophoresis, and then incubates and dries the agarose gel. The isoform gel is visually inspected under UV light. Finally, the densitometer automatically calculates and prints the CK-MB and CK-MM values on the computer screen."

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the isomeric MB pattern in this case was similar to those in AMI patients. This same AMI pattern was observed even in samples that were taken after long periods of time.

CASE REPORT

A 62-year-old woman presented with complaints of chest oppression. ECG revealed T inversion in leads I and aVL (Fig. 1). Routine biochemistry results were all within the normal range, except for elevated values of total CK and LDH. She was admitted for tests to rule out AMI (non-Q type) or severe ischemia (coronary spasm). The patient was a known hypertensive who had been receiving treatment for the previous 12 years. Subsequent laboratory data continued to reveal abnormally high total CK and LDH values for more than six months. One week after admission, the ECG reverted to normal, with no T wave inversion. The patient subsequently had no particular symptoms or complaints. Exercise ECG revealed normal stress capability with no significant ST-T changes. Wall motion and thickness as assessed by echocardiography were normal. Thallium scintigraphy revealed an area of fixed hypoperfusion with reversed redistribution (RD).

CK isomers and isoenzymes, as well as LDH isoenzymes, were separated and analyzed on agarose plates using a rapid analyzer. After 5 ml of the patient’s blood was centrifuged at 3000 rpm for 10 min, serum was removed for immediate assay or stored at -70 °C. No preservatives or activators were added to the sera. The tests were performed immediately or within 4 h of storage. The samples were subjected to high-voltage electrophoresis on agarose gel plates at a low temperature. This ensures a rapid separation of the different CK/LDH bands.14,15 This assay is accurate and requires only 30 min for the entire process. A schematic diagram of the procedure is illustrated in Fig.2. After electrophoresis, the plate was
TABLE I RELEVANT BIOCHEMISTRY DATA

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<td>WBC (6.5±2)</td>
<td>6100</td>
<td>6200</td>
<td>5600</td>
<td>7300</td>
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<tr>
<td>SGOT (10—30 U/l)</td>
<td>38</td>
<td>45</td>
<td>59</td>
<td>63</td>
<td>65</td>
<td>117</td>
<td>42</td>
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<td>SGPT (3—29 U/l)</td>
<td>25</td>
<td>43</td>
<td>58</td>
<td>67</td>
<td>85</td>
<td>58</td>
<td>107</td>
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<tr>
<td>CK (2:34—152</td>
<td>589</td>
<td>769</td>
<td>1335</td>
<td>872</td>
<td>1485</td>
<td>950</td>
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<td>CK-MB 2% (1—4%)</td>
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<td>LDH (224—454 U/l)</td>
<td>436</td>
<td>486</td>
<td>465</td>
<td>477</td>
<td>557</td>
<td>605</td>
<td>731</td>
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<td>ZTT (2—12 U/l)</td>
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<td>13.7</td>
<td>15.3</td>
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<td>15.4</td>
<td>11.5</td>
<td>13.9</td>
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<td>B-LP (200—500 mg/dl)</td>
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<td>591</td>
<td>558</td>
<td>578</td>
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<td>LDL (190—580)</td>
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<td>VLDL (210)</td>
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<td>HDL (2:24—92</td>
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<td>21</td>
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<td>(2:33—81 mg/dl)</td>
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<td>A/G (1.1—1.8)</td>
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<td>IgG (770—1550 mg/dl)</td>
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<td>TP (6.6—8.2 g/dl)</td>
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<td>T-Bil. (0.25—0.95 mg)</td>
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<td>%HBD (92—205 U/l)</td>
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<td>Glucose (64—117 mg/dl)</td>
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<td>Triglyceride (38—193 mg/dl)</td>
<td>219</td>
<td>141</td>
<td>193</td>
<td>210</td>
<td>183</td>
<td>248</td>
<td>247</td>
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<tr>
<td>LDH5 (&lt;2.7%)</td>
<td>9.74</td>
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<td>15.58</td>
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<td>7.36</td>
<td>10</td>
<td>9.4</td>
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<td>Myoglobin (&lt;35 ng/l)</td>
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viewed under UV light before being scanned by a densitometer.

Fig. 3 shows the results of CK isomer, isoenzyme, and LDH isoenzyme analysis in a normal subject, an AMI case, and the present anomalous case. As can be seen from Fig.3a, there is practically no MB isomer band in the normal case. In the MM band, only the MM-1 band is high with low levels of MM bands-2 and 3, the tissue form bands. In the case of a typical AMI patient, MB is clearly elicited, with the MB-2 band higher than the MB-1 band, which is a characteristic pattern in an early AMI case. In the present case, the anomaly band appears between the MM and MB bands. All three of the MM bands are elevated. Finally, the MB band is not only elicited, but also shows the same pattern as that of the AMI patient, with the MB-2 band higher than the MB-1 band. The anomaly band is close to the immediate anodal aspect of the MM band. Fig. 3b shows the isoenzyme patterns. There is no significant difference in the MM bands of the normal subject, the AMI case, and the anomalous case. The MB band appears in both the AMI patient and the anomalous patient. In this case, the anomaly band is not as misleading. The anomaly band could be interpreted as a sub-band, an artifact, or an anomaly band. Fig. 3c shows the LDH isoenzyme patterns in a normal subject, a patient with AMI, and the anomalous case. In the normal LDH pattern, LDH-2 is higher than LDH-1, while LDH-1 is higher than LDH-2 in AMI. In the anomalous case, LDH-1 and 2 show normal patterns, but LDH-5 is elevated in a manner reminiscent of patients with either hepatic congestion (anoxia) secondary to myocardial infarction or primary liver disease. However, this patient's elevated LDH-5 could not be attributed to either of these two conditions. Table I shows relevant biochemistry laboratory data for the past 6 years. Persistently elevated values of total CK, LDH, serum aspartate transaminase (SGOT), serum alanine transaminase (SGPT), as well as zinc turbidity test (ZTT)
and triglycerides can be noted.

Fig. 4 shows the CK isomer/isoenzyme, and LDH isoenzyme values of this anomalous case on different occasions over the past 3 months, showing the same pattern throughout this period. Although protein fraction values were normal for the past 6 years, they were monitored to rule out the possibility that the complex was due to a disorder of different protein fractions.

Fig. 5a shows a graphic representation of the CK, LDH, and SGOT values for the past 6 years. They never touched the baseline level, and all three followed a similar pattern of increase and decrease during different time periods. Fig. 5b compares the values of ZTT and the liver enzymes, SGOT and SGPT. These values also never reached the baseline value in the previous 6 years, and the three values generally followed the same increase-decrease pattern.

DISCUSSION

CK isomers have recently been recognized as highly specific and sensitive parameters in the diagnosis of AMI. Even though ECG still remains the method of choice for all practical purposes, it cannot always be relied upon for the diagnosis of AMI. There are cases of bundle branch blocks, or other atypical ECG patterns, in which the diagnosis of AMI would be difficult to determine solely on the basis of ECG. We have found that isomers are very useful in the diagnosis of non-Q-AMI, in which the ECG fails to elicit ST-T changes and a subsequent Q wave, which are the diagnostic patterns of typical AMI.

However, in this particular patient the isomer band pattern was similar to that typically found in AMI. She had not suffered from any known significant illness in the

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Fig. 5. (a): Chronological changes in the enzymes-CK, LDH, and SGOT during the past six years.

(b): Chronological changes in SGOT, SGPT and ZTT during the past six years.

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past, except for being treated for hypertension. In our experience, this was the first false positive case. The fact that CK-MB is such a cardio-specific marker led us to rule our myocarditis and other related causes of elevated CK, such as muscular dystrophy, pulmonary or cerebral diseases, or skeletal-muscular disorders.

Most previous reports of isoenzyme macroanomaly cases have shown them to be a complex of CK-BB and IgG, or both CK-MB/BB and IgG

However, in this case, the possibility that the anomaly was a complex between CK-BB and IgG was ruled out.

Macro-CK cases also usually have elevated levels of CK-MB isoenzyme. However, macro-CK is not associated with any known pathology.

The long biological life of macro-CK results in prolonged elevated serum CK levels. The possibility that this anomaly was of mitochondrial origin was also ruled out. The CK-mitochondrial complex normally migrates cathodal to CK-MM on electrophoresis. In this case, however, the complex migrated to the immediate anodal aspect of CK-MM.

The possibility that this anomaly was a B-lipoprotein complex was considered, since the level of this protein had always been slightly over the upper normal limit. However, this possibility was also ruled out because there was no B-lipoprotein arc fluorescence during electrophoresis on agarose gel. The elevation of total LDH and its 5th isoenzyme (LDH-5) could be attributed to liver disorder or striated muscle fiber injury. Since AMI was not apparent, elevated LDH-5 due to hepatic injury secondary to AMI was ruled out. Thus, although we examined all of the possible sources of the macroanomaly complex in this patient, we were unable to specifically identify any particular cause. The cardio-specific marker CK-MB was persistently elevated and thallium scintigraphy showed a fixed hypoperfusion area with RD of the anterolateral wall. RD is usually interpreted as an inconclusive finding and reported as normal. Occasionally, it is interpreted as subendocardial-type myocardial infarction (non-Q-AMI). Furthermore, it is debatable whether the increased values of SGOT, SGPT, LDH, and CK are related to cardiac pathology in this case.

However, regardless of whatever type of complex this macroanomaly case may belong to, the important fact remains that if this patient was to have an AMI, the CK isomer pattern would be different from that of a typical AMI case, creating the possibility of misdiagnosis of AMI. On the other hand, it would be more likely for a non-AMI case to be misinterpreted as an AMI case. Thus, an anomaly of this type can detract from validity of using isomers as specific markers for AMI. Further study and research is needed to clearly comprehend the complex background, as well as the related precautions to be taken regarding these type of cases in relation to the diagnosis of AMI. Thus, we conclude that CK isomers are undoubtedly a useful asset for the diagnosis of AMI, especially in doubtful cases such as non-Q AMI and possibly bundle branch block. However, in the presence of a macroanomaly, regardless of its origin, the possibility of false positive or negative results must be kept in mind.

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REFERENCES


6. STEIN W, BOHNER J, EGGSTEIN M: Macro creatine kinases in hospitalized patients. (Abstract)