Immunoglobulin-associated Creatine Kinase Masquerading as Macro-creatine Kinase Type 2 in a Statin User

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Abstract

Macro-creatine kinase (CK) is a cause of falsely elevated CK. Macro-CK type 1 is immunoglobulin-associated CK; type 2 is polymeric mitochondrial-CK. An elderly asymptomatic lady had an elevated CK level after receiving statin therapy. Her CK gel electrophoresis analysis demonstrated coexisting macro-CK type 1 and type 2 patterns. Further analysis by immunofixation and mixing this patient’s serum with CK control material revealed an IgG-associated macro-CK that mimicked the electrophoretic pattern of macro-CK type 2. This highly unusual discovery suggests the possibility of the misinterpretation of macro-CK type 1 as macro-CK type 2. Falsely elevated CK is still common despite modern laboratory instrumentation and should be investigated.

Key words: creatine kinase, immunoglobulin-associated, isoenzyme, macro-creatine kinase, mitochondrial creatine kinase


Introduction

Creatine kinase (CK; EC 2.7.3.2) is an enzyme that catalyzes the reversible phosphorylation of creatine by ATP. It is a dimeric enzyme that forms 3 different isoenzymes: CK-BB, CK-MB, CK-MM, by the alternate pairing of M and B subunits. Owing to its metabolic importance, CK can be found in a wide variety of tissues, although the highest activities are found in skeletal muscle, brain and heart (1).

Statin is a potent low-density lipoprotein lowering agent, which can induce myopathy that ranges from myalgia to rhabdomyolysis (2, 3). Currently, measurement of total CK activity is recommended for the diagnosis and subsequent monitoring of muscular adverse effects of statin therapy (4).

Macro-CK is an abnormal form of CK, which can falsely elevate CK measurement. The false elevation can variably occur with total CK or CK-MB alone, or in combination (5). Macro-CK type 1 (molecular weight >200 kDa) is an immunoglobulin (Ig)-associated CK complex while macro-CK type 2 (molecular weight >300 kDa) is a polymeric complex of mitochondrial-CK (6). We describe a case of persistent macro-CK type 1 that is associated with immunoglobulin G, in whom the electrophoresis pattern mimics that of macro-CK type 2.

Case Report

A 79-year-old woman with a background history of diabetes mellitus, hypertension and previous myocardial infarction was found to have CK activity of 1,144 U/L (reference interval: 20-300 U/L) on routine monitoring after the initiation of statin therapy. Her serum aspartate aminotransferase and alanine aminotransferase activities were not elevated. She was in apparent good health and good spirit. She denied suffering from muscle ache, muscle weakness or chest discomfort. Other muscle enzymes such as lactate dehydrogenase and aldolase were within the reference limits. Her troponin I concentration was likewise not elevated.

The serum of the patient was subjected to electrophoretic
Progression of the creatine kinase electrophoresis pattern of the patient over time. Lane 1 (Control) shows the positions of the creatine kinase-MM, -MB, and -BB isoenzymes. The subsequent four lanes show the evolution of the creatine kinase electrophoresis pattern of the patient between August 2008 and May 2010.

Serum of the patient was immunofixed with antisera to heavy chains of IgG (G), IgA (A), IgM (M), and Kappa (K) and Lambda (L) light chains after electrophoretic separation. Intense bands were visualized at IgG heavy chain and kappa light chain lanes after immunofixation and substrate-chromogen incubation. CK isoenzyme control material from Sebia Hydrasys Hydragel ISO-CK kit was included as the control in the same run, without being subjected to antiserum (ELP).

Three distinct bands were seen on the agarose gel, none of which corresponded to the CK isoenzyme controls included in the run (Fig. 1a). Two bands (densitometric fraction of 21.5% and 7.5%) were visualized between the CK-MB and CK-MM fractions, while an intense third band (71%) was seen cathodic to CK-MM fraction. The electrophoresis pattern observed was consistent with type 1 (between CK-MB and CK-MM) and type 2 (cathodic to CK-MM) macro-CK band positions, respectively. Two years after the initial diagnosis, a fourth band anodic to the CK-MB control appeared (Fig. 1a). The apparent reduction in the intensity of the macro-CK type 1 bands in May 2010 may represent the early phase of the gradual disappearance of the macro-CK
type 1. The patient remained clinically well throughout the period of follow-up.

To identify the immunoglobulins associated with the CK enzyme of the patient, a hybrid method combining immunofixation electrophoresis and substrate-chromogen development similar to this (7) was performed. Briefly, 500 μL of serum from the patient was incubated for 10 minutes at room temperature with 5 μL of activation solution (Hydragel ISO-CK). Subsequently, the activated serum was electrophoresed and immunofixed using the Hydrasys Hydragel 4 IF (Hydragel ISO-CK). Subsequently, the activated serum was electrophoresed and immunofixed using the Hydrasys Hydragel 4 IF assay (Sebia, France), according to instructions from the manufacturer. After the required immunofixation incubation time, the gel was immediately incubated with CK substrate-chromogen mixture (Hydragel ISO-CK) in a water bath at 37°C for 30 minutes. Reaction stopping agent was added and incubated for another 10 minutes at 37°C before the gel was dried and washed. CK isoenzyme control material from Sebia Hydrasys Hydragel ISO-CK kit was included as control in the same run, without being subjected to antiserum. The lanes containing IgG heavy chain and kappa light chain antisera had one intense band each at the gamma region, indicating the presence of IgG-bound macro-CK (Fig. 1b). Both bands migrated significantly slower than the CK isoenzyme control in the first lane.

To determine the CK isoform associated with the immunoglobulins, the patient’s serum was incubated with the CK isoenzyme control material of the Sebia Hydrasys Hydragel ISO-CK assay, similar to an experiment previously described (7). Here, 20 μL of the patient’s serum was mixed with 180 μL of the control material and incubated for 6 hours at 4°C. The mixture was then subjected to routine CK isoenzyme electrophoresis as described above. The densitometer scan of the agarose gel of the incubated serum-control mixture showed significant modification of the mobility of the CK-MM isoenzyme control, resulting in an electrophoretic pattern resembling that of the patient and appearance of macro-CK type 2-like banding pattern (Fig. 2). This indicates that the anti-CK immunoglobulins in the serum of the patient might have interacted with the CK-MM isoenzyme present in the control solution, and retarded the electrophoretic mobility of the complex formed.

This study was exempted from the institution review board of the National University Hospital, Singapore.

Discussion

Macro-CK is frequently associated with certain diseases but can also be found in apparently healthy individuals. Macro-CK type 1 occurs most commonly in patients with myopathy, heart disease, gastrointestinal disease and autoimmune disease (8, 9). Macro-CK type 2 is more commonly associated with patients with malignancies and those who are gravely ill (6, 10). Nevertheless, the possibility of malignancy was not ruled out in the present patient as she had declined further investigations. In this patient, the asymptomatic moderate elevation of CK activity was caused by the presence of macro-CK type 1, which had several unusual features.

Macro-CK type 1 is the most frequently formed between CK-BB and IgG, and less frequently with IgA or IgM (6). The hybrid immunofixation and sera-control mixture studies (Fig. 1b) suggested that the macro-CK found in this patient was formed between CK-MM isoenzyme and anti-CK IgG.

CK isoenzyme that migrates anodic to the CK-MB has been seen in patients with metastatic prostate cancer, which eventually disappeared several months later, and has been suggested as a variant of CK-BB or CK-MB isoenzyme (11). The presence of a band cathodic to the CK-MM suggested the possibility of coexisting macro-CK type 1 and macro-CK type 2. However, the appearance of a retarded CK-MM control mobility after prolonged incubation with the serum of the patient, and the clinical features of patient made the above two scenarios unlikely.

To our knowledge, this was the first time an immunoglobulin-associated CK was found not migrating at the typical type 1 position between CK-MM and CK-MB. Instead, the immunoglobulin-bound CK was found migrating at the position typical of macro-CK type 2. These findings opened the possibility of misinterpretation of macro-CK type 1 as macro-CK type 2.

Macro-CK is a relatively common phenomenon, being
present in up to 2.6% of patient samples (6). It remains a cause of spuriously elevated serum CK activity even with modern day instrumentation (5). It is possible that physicians may encounter this phenomenon when measuring the serum creatine kinase activity of a patient. Hence, asymptomatic elevation or clinically discrepant CK measurements should alert the physicians to the possibility of the presence of macro-CK. Joint investigation between the laboratory and the physician should be undertaken to resolve the discrepancy, and avoid mismanagement of the patient.

Macro-CK is generally associated with moderate elevation of total CK activity (6). Under most circumstances, macro-CK is not expected to adversely impact the administration of statin therapy, since the therapy cessation threshold is ten-fold the upper limit of CK reference interval (4). Nevertheless, unrecognized macro-CK may subject a patient to unnecessary repeat testing of CK activity, as patients with CK elevation of between three and ten times the upper reference limit require weekly CK measurement and close monitoring of symptoms (4).

In summary, the present case showed an unusual CK-MM macro-CK type 1 that was associated with the IgG, which also mimicked the electrophoretic pattern of macro-CK type 2 in an apparently well patient receiving statin therapy.

The authors state that they have no Conflict of Interest (COI).

References


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