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CK-MB activity and hemolysis: Where the interference begins?

[CK-MB aktivitesi ve hemoliz: İnterferans nerede başlar?]

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ABSTRACT

Although the positive interference between the CK-MB activity and hemolysis have been previously documented, no knowledge about this interference is present either in the literature, or in the content of the test kit of CK-MB. Thus, the current study aimed to determine the linearity of the positive interference between the CK-MB activity and hemolysis, as well as to determine the effects of adenosine monophosphate and diadenosine pentaphosphate present in the reagent content on the interference of adenylate kinase originated from the erythrocytes. Two different hemolysates with two different hemoglobin concentrations were prepared from the blood samples drawn from the patients for "whole blood counting". Serum pools with normal CK and CK-MB activity and without residual erythrocytes were prepared and separated into aliquots. These aliquots were mixed with the hemolysates of the different concentrations. CK- MB activity was determined by using the immunoinhibition method in these aliquots. A positive and significant relationship was found between mild to moderate hemoglobin concentrations and increasing CK-MB activities ($r = 0.982$, $p < 0.0001$). CK-MB activity measurements by the immunoinhibition method have been found to be interfered with the hemolysis and to be positively correlated with the severity of hemolysis. It was found that the positive interference begins in low hemoglobin concentrations and increases positively with hemoglobin concentrations. Adenosine monophosphate and diadenosine pentaphosphate, have been shown to prevent the interference of mild to moderate hemolysis for the determination of CK activity in the serum. These inhibitors did not have the same effect on CK-MB activity that is lower than CK activity.

Key Words: Interference, Hemolysis, CK-MB, Immunoinhibition

ÖZET

CK-MB aktivitesi ve hemoliz arasındaki pozitif interferans çok iyi bilinmekle birlikte bu interferansın nerede başladığı test kitinin içeriğinde ve literatürde belirtilmemiştir. Bu nedenle CK-MB aktivitesi ve hemoliz arasındaki interferansın linearitesini belirlemeyi ve eritrositlerden açığa çıkan adenilat kinazın interfere edici etkisine karşı reaktif içeriğinde bulunan adenozin monofosfat ve diadenozin pentafosfatın etkinliğini araştırmayı amaçladık. Tam kan sayımı için alınmış örneklerden iki farklı hemoglobin konsantrasyonuna sahip iki hemolizat hazırlandı. Normal CK ve CK-MB aktivitesine sahip ve rezidüel eritrosit içermeyen serum havuzu hazırlandı ve analizlenmek üzere kısımlara ayrıldı. Bu ayrılan kısımlar farklı konsantrasyonlarda hemolizatlar ile karıştırıldı. CK-MB aktivitesi immunoinhibisyon metodu kullanılarak belirlendi. Hafif hemolizden şiddetli hemolize kadar, değişik hemoglobin konsantrasyonları ile CK-MB aktiviteleri arasında pozitif ve anlamlı korelasyon bulundu. ($r = 0.982$, $p < 0.0001$). İmmunoinhibisyon metodu ile yapılan CK-MB aktivite tayininde aktivite hemolizin şiddeti ile pozitif korelasyon gösterdi. Pozitif interferans, düşük hemoglobin konsantrasyonlarında görülmeye başladı ve hemoglobin konsantrasyonu arttıkça CK-MB aktivitesinin arttığı gözlendi. Adenozin monofosfat ve diadenozin pentafosfatın, hafiften orta dereceye kadar olan hemolizde serum CK aktivitesi için interferansı önlediği görülmekle beraber bu inhibitörlerin, CK aktivitesine göre daha düşük düzeyde bulunan CK-MB aktivitesi için aynı etkiyi göstermediği bulundu.

Anahtar Kelimeler: İnterferans, hemoliz, CK-MB, immunoinhibisyon

INTRODUCTION

Creatine kinase-MB (CK-MB) is one of the most-often used cardiac markers for the diagnosis of acute myocardial infarction. CK-MB activity, or mass assay, is frequently required for the differential diagnosis of acute chest pain. However, there are some factors that affect CK-MB activity [1].

One well-known factor encountered in clinical laboratories and the source of artifactural values for CK-MB activity is hemolysis [2]. Hemolysis is shown by visual evidence when hemoglobin concentrations are higher than 20 mg/dL [2,3]. Measurement of CK and CK-MB activity are significantly increased by hemolysis, although erythrocytes do not contain creatine kinase activity [4,5]. This effect of hemolysis has two causes: First, adenylate kinase and glucose-6-phosphate dehydrogenase enzymes are released into serum from erythrocytes. Adenylate kinase increases ATP (adenosine triphosphate), which is a subproduct in the immunoinhibition process. ATP and glucose-6-phosphate play a role as substrates in CK (creatine kinase) and CK-MB reactions (Figure 1), and the high levels of the substrates in the serum result in increased CK-MB activity [5,6].

The second reason of interference is that hemolysis increases the absorbance at the short wavelength of the visible spectrum (300-500 nm) [2]. In recent autoanalyzers, measurements are done at two wavelengths and prevent this effect.

Measurement of CK-MB with the immunoinhibition method is simple, fast and also more commonly used. In measuring activity of CK-MB by this method, CK-M subunits are inhibited without being affected by CK-B catalytic activity. The CK-B activity after CK-M subunit inhibition is half of the CK-MB activity. The CK-BB isoenzyme is in very low concentrations under normal conditions (less than 5 U/L). Therefore, twice the CK-B activity is equivalent to the CK-MB activity [7,8].

CK-MB activity is measured in our laboratory by the immunoinhibition method (Roche Diagnostics, cat No.: 1929011 and Olympus System Reagent, cat No.: OSR6153), with PP Modular (Roche Diagnostics GmbH, Mannheim, Germany) and AU800 autoanalyzers (Olympus Diagnostica GmbH, Ireland). Both products contain equal amounts of adenosine monophosphate (AMP) and diadenosine pentaphosphate (Ap5A) as inhibitors of adenylate kinase.

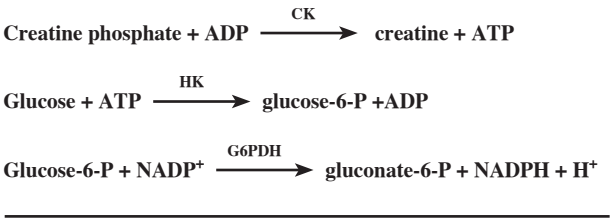

Figure 1: Steps of the CK and CKMB activity determinations (formation rate of NADPH is proportional to the CK or CKMB activity).

Interference of CK-MB by hemolysis is discussed in the test prospectus (instructions) for the test kit. The prospectus of CK reactive (Roche Diagnostics, cat No.: 1552147 and Olympus System Reagent, cat No.: OSR6179) also indicates that the CK measurements will not be affected by hemolysis until the hemoglobin concentration reaches to 200 mg/dL. The literature also supports the fact that CK results will not be affected by mild hemolysis using the creatine kinase N-acetyl cysteine (CK-NAC) method, because erythrocytes do not contain CK activity. Otherwise, moderate and severe hemolysis will interfere the results through the adenylate kinase activity [4].

Although the effect of degree of hemolysis on CK-MB measurements by the immunoinhibition method is not mentioned in the literature, under normal conditions, it is possible that CK-MB activity can easily be affected because it has a lower concentration in serum than does CK.

In this study, we aimed to determine the linearity of the positive interference between CK-MB activity and hemolysis, and the effect of AMP and Ap5A in the reagent content against the effect of interference of adenylate kinase from erythrocytes.

MATERIALS AND METHODS

Two "whole blood counting" sample pools drawn from different patients using tubes containing EDTA (BD Vacutainer, UK) were used in the study. The plasma was separated by centrifugation the sample at 2500 × g for 10 minutes. After addition of a 0.9% NaCl solution, the erythrocyte suspension was recentrifuged at 2500 × g for 10 minutes. After the second centrifugation, the liquid fraction was excluded, 0.9% NaCl solution was added to rest of the sample, and the centrifugation was repeated. After removing the liquid fraction, the erythrocyte cells (without plasma) were stored at -20°C for 1 day. Hemolysates were stored at room temperature.

Hemoglobin was measured for 3 times using the Cell-Dyn 3700 analyzer by a spectrophotometric method. Hemoglobin concentrations were found as 8.78 g/dL in the first hemolysate and 5.7 g/dL in the second hemolysate. Additionally, a serum pool with 10 U/L CK-MB activity was prepared. This pool was centrifuged at 2500 × g for 10 minutes to remove the residual erythrocytes. The hemoglobin concentration in the serum pool was measured as 1 mg/dL using sodium carbonate as a working solution in a spectrophotometer (Schimadzu UV-1208, Japan) [9]. Twenty aliquots (100 µL) were prepared from this pool. One hundred microliters of 8.78 g/dL hemolysate (8780 mg/dL) were mixed with the first aliquot. One hundred

microliters of this was mixed with the second aliquot, and 100 µL of this second aliquot was mixed with a third aliquot. This was repeated for 10 aliquots to obtain samples that had half the hemoglobin concentrations for each dilution. This also was repeated for the remaining 10 aliquots with 5.7 g/dL (5700 mg/dL). CK-MB activity was measured twice for each of the 20 samples. Activity was determined by using immunoinhibition method (Roche Diagnostics, cat No. 1929011) with PP Modular autoanalyzers (Roche Diagnostics GmbH, Mannheim, Germany). All data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 11.0, SSPS Inc, Chicago, Ill, USA).

RESULTS

The CK-MB activities of the different hemoglobin concentrations are shown in the Table 1. The hemoglobin concentration in the first aliquot was decreased by half after addition of an equal amount of hemolysate. The hemoglobin concentrations of subsequent aliquots were also decreased by half due to dilution. There was a positive and significant relationship between the hemoglobin concentrations and CK-MB activities ($r = 0.982$, $p < 0.0001$) (Figure 2).

Table 1: CK-MB activities of 20 samples that were prepared from two different hemolysates having different hemoglobin concentrations.

Aliquots prepared from the hemolysate of 8780 mg/dL		**Aliquots prepared from the hemolysate of 5700 mg/dL**	
Hb (mg/dL)	CK-MB (U/L)	Hb (mg/dL)	CK-MB (U/L)
4390	1012	2850	496
2195	375	1425	227
1097.5	183	712.5	108
548.7	105	356.3	76
274.4	60	178.1	50
137.2	53	89.1	39
68.6	41	44.6	35
34.3	35	22.3	31
17.1	31	11.2	30
8.6	30	5.6	22

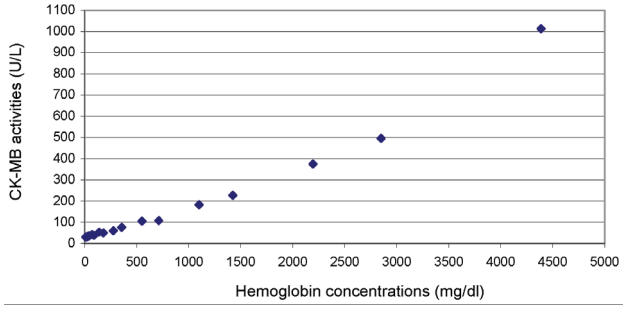

Figure 2: CK-MB Activities versus hemoglobin concentrations in the samples ($n = 20$, $r = 0.982$, $p < 0.0001$).

DISCUSSION

Adenylate kinase was reported as the main reason for interference of hemolysis on CK and CK-MB activities [4,6]. Interference of hemolysis can be prevented by addition of AMP and Ap5A, the competitive inhibitors of adenylate kinase, into the reactive content [4]. The appropriate combination of AMP and Ap5A may inhibit the adenylate kinase of the erythrocyte [10].

Roche Diagnostics, GmbH, has added the AMP and Ap5A (Reagent 1) into the reactive solution as inhibitor. By using these inhibitors, mild hemolysis is suggested to be tolerated for accurate measurement of CK-MB and CK activity. However, the results of this study, showed that mild hemolysis continues to positively interfere with CK-MB results. We speculate that all reagent kits and devices using the immunoinhibition method will cause a positive false result for the determination of CK-MB activity because of this mild hemolysis.

The immunoinhibition method is an easy, fast, and sensitive method that is preferred worldwide. However, there are many factors that interfere with this method. Hemolysis is only one of these factors. Other factors that will cause increased CK-MB activity include type 1 macro CK that causes false positive results in the immunoinhibition method and some organ disorders (eg, lung tumors, gastrointestinal disorders, central nervous system neoplasm, and tumors of the kidney, prostate bladder, testes, breasts, uterus, and ovaries) that contain the CK-BB isoenzyme in high concentrations, [4, 5]. Fortunately, the cardiac determinant of acute myocardial infarction is not dependent only on CK-MB activity. CK-MB mass measurements are not affected by mild hemolysis, macro CK forms, and disorders that cause CK-BB isoenzyme increases, and do not interfere with the results [7,11,12]. Mass measurements are more sensitive than activity measurements and can determine the serum abnormalities earlier [7,13]. Troponin I is a superior determinant that increases later, but only in the heart muscle, and is valuable in the diagnosis of acute myocardial infarction [14].

In conclusion, this study demonstrated that CK-MB activity measurements by the immunoinhibition method could be interfered with the hemolysis and are positively correlated with the severity of hemolysis. Positive interference begins in low hemoglobin concentrations and increases positively with hemoglobin concentrations. This method may be eliminated in the future owing to the many factors that interfere with the assay.

List of Abbreviations

AMP	: Adenosine monophosphate
Ap5A	: Diadenosine pentaphosphate
ATP	: Adenosine triphosphate
CK	: Creatine kinase
CK-MB	: Creatine kinase-MB
CK-NAC	: Creatine kinase N-acetyl cysteine
EDTA	: Ethylenediaminotetraacetic acid

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