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Research Article

Intravenous cannula can increase serum creatine kinase MB activity

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Abstract

Objectives: Different blood sampling methods may result in differing creatine kinase MB (CK-MB) results. The aim of this study was to assess the effect of 2 phlebotomy methods on the CK-MB level in serum samples of healthy adults.

Methods: The study used 50 volunteers (20 females, 30 males) who were employees at Baskent University Hospital in Ankara, Turkey. The subjects were randomly assigned to the study group. Blood samples were collected with the most widely used equipment for each method being tested (20G intravenous [IV] cannula and 20G needle unit) to compare the effects of different methods of blood sampling. The mean values of CK-MB and creatine kinase (CK) activity were compared using a paired samples t-test.

Results: The mean CK-MB activity was 14.4±4.1 U/L and the mean CK activity was 127.3±17.1 U/L in samples that were drawn with a 20G needle unit, while in samples drawn with a 20G IV cannula, the corresponding results were 19.3±3.8 U/L and 132.5±16.1 U/L ($p<0.001$ for both).

Conclusion: The study results indicated that blood sampling using an IV cannula caused more mechanical injury to vessel endothelium than sampling with a needle unit. This translates to greater interference with CK-MB and CK activity when the cannula method is used. Blood sampling with a cannula led to elevated CK-MB activity.

Keywords: Blood sampling, creatine kinase MB, hemolysis, interference, phlebotomy

Cardiac emergencies can be a cause of greater hospital mortality [1]. The level of creatine kinase MB (CK-MB) isoenzyme activity in serum is still an important marker in the differential diagnosis of acute chest pain and the planning of treatment for cardiac cases in hospitals where troponin and CK-MB mass tests cannot be performed [2]. However, different blood sampling methods used in the emergency room and the blood-sampling unit of the hospital laboratory may result in differing CK-MB results [3]. For example, when a patient with chest pain arrives at the emergency department, an intravenous (IV) cannula is immediately inserted and blood is drawn via this catheter. There are various factors that affect CK-MB activity. Even if the chest pain is not related to a cardiac etiology, the CK-MB activity value may be high with this collection method for other reasons, such as hemolysis. In some cases, CK-MB activity measured using an immunoinhibition technique may exceed the upper-normal limit, whereas CK-MB mass measurements of the same sample may be in the normal range. Many laboratories still use immunoinhibition as the routine method to measure CK-MB activity in serum for several reasons [4, 5]. In Izmir, the third largest city in Turkey, about 100.000 CK-MB activity tests per year are performed in 15 different state hospitals.

Hemolysis before blood analysis is a source of error in all clinical laboratories and may occur if the hemoglobin (Hb) concentration in a sample is greater than 27 mg/dL [6]. Research has shown that measurements of hemolytic samples have indicated higher levels of CK-MB activity than actually existed in the sample (i.e., positive interference, depending on the intensity of hemolysis), and lower measurements of CK-MB mass (neg-

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ative interference) [7]. It has also been established that hemolysis exaggerates measurements of creatine kinase (CK) as well as CK-MB activity, and this is interesting since erythrocytes do not contain CK [8]. Two primary theories for this effect have been proposed. One suggestion is related to the passage of adenylate kinase, adenosine triphosphate (ATP), and glucose-6-phosphate from erythrocytes into the serum when these cells rupture. In the immunoinhibition method of blood testing, the action of released adenylate kinase produces high levels of ATP as a by-product. Both ATP and glucose-6-phosphate are substrates in CK and CK-MB reactions [9]. The second theory is that hemolysis increases the absorbance of light in the short wavelength of the visible spectrum (300-500 nm) [10, 11]. Such a change in absorbance would alter laboratory results.

The needle sizes most commonly used for drawing blood are gauges 19-22 (outside diameter 1.06-0.71 mm). Hemolysis is usually less severe when blood is drawn through a small-bore needle because there is less blood turbulence than with the use of a larger-bore needle [12].

The aim of this study was to assess how blood sampling phlebotomy methods affect CK and CK-MB levels in serum samples drawn from healthy adults.

Materials and Methods

The study included 50 volunteers (20 females and 30 males) who were employees at Baskent University Hospital in Ankara, Turkey. The average age of the volunteers was 30.3±4.11 years (23-40 years). The Baskent University Faculty of Medicine Clinical Research Ethics Committee approved the protocol (No: KA03/26). The subjects were randomly assigned to this study group. Blood samples were collected with the most widely used equipment for each method being tested (20G IV cannula and 20G needle unit) to compare the effects of different methods of blood sampling. A single phlebotomist performed all of the sampling, and the collections were performed in the blood-sampling unit of the study laboratory. Each specimen was 5 mL in volume and was drawn into a simple, plain biochemistry tube (BD Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Veins of similar diameter were used for all of the sampling, and the blood was drawn under conditions of minimal venous stasis. Each subject had 2 samples collected: the first from the right brachial vein, using a 20G IV cannula (BD Venflon, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and a second from the left brachial vein, using a 20G needle unit. After each sample was obtained, the blood was centrifuged at 2500xg for 10 minutes at room temperature. Then 1 mL of serum was separated off, and this was centrifuged for another 10 minutes at 2500xg at the same temperature. The activity level of CK and CK-MB was then analyzed using a PP Moduler autoanalyser (Roche Diagnostics, Basel, Switzerland). Serum CK activity was measured according to the CK N-acetyl cysteine (CK-NAC) method (CK-NAC Reagent, No. 1552147; Roche Diagnostics, Basel, Switzerland), using the guidelines of the International Federation of Clinical Chemistry and the German Society for Clinical Chemistry [13]. Serum CK-MB activity was measured using the immunoinhibition method (CKMB Reagent, No. 1929011; Roche Diagnostics, Basel, Switzerland) [14]. The normal range of the CK test was 0-165 U/L for women and 0-190 U/L for men, and the CK-MB test was 0-25 U/L for both sexes. Intra-assay and inter-assay coefficient of variation (CV%) measurements were performed for the CK and CK-MB tests. The ratio of CK-MB to CK was also calculated. The serum Hb concentration was measured with a Shimadzu UV-1208 spectrophotometer (Shimadzu Corp., Kyoto, Japan) with sodium carbonate working solution [15]. The rejection criteria of the study were hemolyzed samples and increased CK activity. Visual hemolysis was not observed in the serum samples. Each subject's serum total CK activity was in the normal range.

Statistical analysis

The data were analyzed using SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, IL, USA). CK, CK-MB, and Hb results were reported as mean±SD. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess normality. The mean values for CK activity, CK-MB activity, and serum Hb concentration in the study group were compared using a paired samples t-test.

Results

The mean CK activity was 127.3±17.1 U/L, the mean CK-MB activity was 14.4±4.1 U/L, the ratio of CK-MB to CK was 11.3±2.7%, and the mean serum Hb concentration was 7.3±4.0 mg/dL in the samples drawn with a 20G needle unit. In the same group, in the samples drawn using a 20G IV cannula, the corresponding results were a mean of 132.5±16.1 U/L, 19.3±3.8 U/L, 14.6±2.4%, and 7.5±4.0 mg/dL. Comparisons revealed significant differences between the CK results, the CK-MB results, and the ratio of CK-MB to CK ($p<0.001$ for all). However, there was no significant difference in the Hb results in the group ($p>0.05$) (Table 1). Intra-assay CV measurements for CK and CK-MB tests were 1.21% and 3.99%, respectively. Inter-assay CVs for CK test in low- and high-level measurements were 1.93% and 1.55%, respectively (mean inter-assay CV: 1.74%). The inter-assay CV values for the CK-MB test were 4.19% and 2.48%, respectively (mean inter-assay CV: 3.34%).

Discussion

CK-MB is used as a marker for diagnosing the cause of chest pain, and CK-MB activity tests are still frequently used in district hospitals with low patient capacity and limited facilities. However, certain factors restrict the accuracy of this test. The level of CK-MB activity can be falsely elevated in the setting of hemolysis, hypothyroidism, uremia, muscle trauma and the presence of macro CK, and the presence of elevated CK-BB [16-19]. If a blood sample for CK-MB analysis is hemolyzed, a new one should be obtained. However, repeat sampling is

Table 1. Laboratory findings using two methods of blood sampling

	Blood sampling with 20G needle unit (n=50) mean±SD	Blood sampling with 20G IV cannula (n=50) mean±SD	p
CK-MB (U/L)	14.4±4.1	19.3±3.8	<0.001
CK (U/L)	127.3±17.1	132.5±16.1	<0.001
CK-MB/CK ratio (%)	11.3±2.7	14.6±2.4	<0.001
Free hemoglobin (mg/dL)	7.3±4.0	7.5±4.0	>0.05

CK: Creatine kinase; CK-MB: Creatine kinase MB

not performed in all cases with hemolytic samples because of the rapid turnaround time necessary for prompt diagnosis of myocardial infarction [8]. In this study group, the CK and CK-MB levels in the samples collected with a 20G IV cannula were higher than that seen in the samples collected with a needle unit. Also, the Hb concentration in the blood drawn with the IV cannula was higher than those drawn with the needle units, although the difference in the Hb level was not statistically significant. This may have been due to the gentle conditions of the blood draw. The findings indicate that erythrocyte damage is not the only factor that affects CK and CK-MB levels in serum samples. The physical structure of IV cannulae and/or deeper penetration in the vein may cause damage to vessel integrity. Kinases released from endothelial cells can interfere with CK and CK-MB. In our comparison of sampling using a 20G cannula and a 20G needle unit, all of the tests showed higher levels of CK and CK-MB with the cannula method. In addition, higher Hb concentrations were detected in blood collected via IV cannula than in blood collected with a needle unit. These results are consistent with findings in the international literature, which have reported that blood collection by IV catheter leads to more hemolysis [3, 12, 20]. Examination of the results indicates that the false-positive effect of hemolysis is much greater on CK-MB activity than CK activity. This is not unexpected for CK-MB activity because the level of this enzyme in the serum is lower than the level of the CK enzyme. We concluded that mild to moderate hemolysis interferes with CK-MB results more than CK results. This result is partially parallel to literature reports indicating that mild hemolysis will not affect the measurement of CK activity [21]. However, mild hemolysis significantly interfered with CK-MB activity measurement according to the results of this study. The primary weakness of this study is that a marker (for example, vasoactive mediators of endothelium origin) was not examined to show vascular endothelial damage. Studies measuring vessel endothelial damage can further explain the mechanisms of non-hemolysis interference in enzyme measurements such as CK-MB.

Conclusion

In conclusion, hemolysis in a blood sample may falsely exaggerate CK-MB levels and thus mislead a physician who is trying to diagnose a patient with chest pain. To determine whether a patient actually has elevated serum CK-MB activity, care should be taken to avoid hemolysis during blood sample collection, transportation, centrifugation, and examination in the laboratory. Furthermore, measures to exclude hemolysis can be implemented prior to analysis. Our study results showed that blood sampling by IV cannula caused much more mechanical damage to erythrocytes and vessel endothelium than sampling with a needle unit. There was greater interference with CK-MB activity when the cannula method was used.

Conflict of interest: There is no conflict of interest.

Ethics Committee Approval: This research was approved by the Baskent University Faculty of Medicine Clinical Research Ethics Committee (No: KA03/26).

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