EFFECT OF THE STORAGE ON THE AVTIVITIES OF THE ENZYMES CREATINE KINASE AND LACTATE DEHYDROGENASE IN SERUM AND BLOOD

Entessar Abdul-Lateef*

ABSTRACT:

Objective:

This study was carried out to investigate the effect of storage on the activities of the enzymes creatine kinase (CK) and lactate dehydrogenase(LDH).For this purpose we measured the levels of CK and LDH activities in the blood and serum samples ,stored at 4C° ,during 0 time, and 24 hour.

Method:

Total of 51 subjects (32 males and 19 females),mean age was 20 years (range:18-22years) were selected for the study. They were non-smoker, apparently ,healthy persons, their weights within normal range, and they had no family history of diseases and no drug had been taken in last week. Blood samples were collected after an average fasting for 12 hours, and the level of the enzymes creatine kinase (CK) and lactate dehydrogenase(LDH) were determined immediately (i.e the zero time) and then later after 24 hours .All samples were stored at 4C°.

Results:

In serum sample and during 24 hours of storage ,at 4C° the levels of the CK and LDH enzymes approximately remained constant. In plasma, analysis was made immediately after blood collection and after 24 hours . ASignificant increased in the levels of CK and LDH enzymes activities .

Conclusion:

Based on these results, we can conclude that a contact between blood cells and plasma stored at 4C° for an overnight ,my produce changes in the activities of CK and LDH enzymes.

Key words:Storage,blood ,serum. Enzymes activities

INTRODUTION:

Laboratory tests are used by clinicians for diagnosis, monitoring, and prognosis in patients with different diseases. A number of factors, primarily preanalytical and analytical or normal biological variations affect the accuracy of test results.

Preanalytical factors such sample collection and handling, diet, exercise and drugs can all impact a test result. The key characteristics of any test are its bias and imprecision. Bias is primarily an analytical characteristic, in which reported results

* Department of Biochemistry ,College of Medicine - University of Basrah, Basrah, Iraq.

differ from the actual value. Imprecision, or lack of reproducibility, is due to both physiological and analytical factors [1,2]. Prolonged contact of serum with the clot can cause preanalytical variation. The optimum time interval between sample collection and separation of serum from the clot should be long enough to allow complete clot formation but be shorter than the time in which a significant change in test result is induced by serum-clot contact[3] . The minimum clotting time suggested by the Tietz Textbook of Clinical Chemistry[4] is 20–30 min . During a prolonged contact time between serum and clot, both biological activity of the cells and trans-membrane diffusion can change the concentrations of certain analytes in the serum. NCCLS Procedures for the Handling and Processing of Blood Specimens([5] recommends that serum or plasma should be physically separated from contact with cells as soon as possible, unless conclusive evidence indicates that longer contact times do not contribute to result inaccuracy. A maximum limit of 2 h from the time of collection to the time of separation was also recommended. In medicine it is recognized that blood cell constituents can interfere directly with the measurement of analytes;for example measurements of creatine kinase(CK) activity may be falsely increased by adenylate kinase released from erythrocytes and free haemoglobin may interfere with assessment of bilirubin and optical methods of assessment of other analytes[6].The concentration of lactate dehydrogenase (LDH) ,aspartate aminotransferase(AST)and potassium(K) in erythrocytes is substantially greater (20-160 times) than in plasma; and haemolysis may be expected to cause increases in these analytes[7][8]. The aims of the present study was to assess the effects of the storage at 4C° for 24 hour on the measurement of the activities of the enzymes creatine kinase (CK) and lactale dehydrogenase (LDH) in serum and blood.

METHOD:

A total of 51 (32 males and 19 females),mea of age was 20±2 years (range:18-22years),were selected for the study. They were non-smoker, apparently healthy persons, their weights within normal range, and they had no family history of diseases and no drug had been taken in last week. The participants were mainly selected from undergraduate students at the College of Medicine ,University of Basrah. Ten ml of venous blood -samples after an average fasting of 12hours were drawn through a sterile disposable syringe. Serum was immediately separated by low -speed -centerifugation,and the activities of CK and LDH enzymes were determined immediately (i.e the zero time) and then later after 24 hours of storage at 4C°,other samples of 10 ml were drawn by venipancture for the determination of the activities of the CK and LDH enzymes after contact between red blood cells and plasma for zero and 24 hours .All samples were stored at 4C° .The biochemical parameters were performed in the Department of Biochemistry ,College of Medicine, University of Basrah.The activities of CK and LDH enzymes in serum and plasma were determined by standard methods using kits from Biolabo ,France. The results values were presented as mean±SD.Student "t" test was used for comparison of data. For all analysis, value of 0.05 was considered significant.

RESULT:

Characteristics of all subjects who participated in this prospective study are

summarized in Table 1. The effect of the storage on the activities of the enzymes CK and L DH in a serum sample is presented in Table 2.During 24hour s of storage at 4C°,,the levels of CK and LDH enzymes are approximately remained constant. Table 3 shows the effect of the storage at 4C° on the activities of the enzymes CK and LDH in the plasma in contact with RBC for the period of 24 hours . A significant increase in the activities of the CK and LDH enzymes (P0.01) observed during the storage of whole blood in plane tube at 4C°.This increase was approximately more with the increase in the storage of time(i.e24hours).

DISCUSSION:

The purpose of this study was to examine the differences in the activities of CK and LDH enzymes between blood specimens stored as whole blood and those stored as serum in a controlled laboratory setting using the blood of healthy subjects. By examining the data in Table 2 we can observe that in serum samples ,the activities of the enzymes CK and LDH were not affected by time ,since their levels remained approximately constant, confirming the result of other research[9,10,11]. In the blood (Table 3) ,it is a significant different in the activities of the enzymes CK and LDH from serum, the results of this study were agreement with the results of other investigators [12,13,14] .The reason for this difference between blood and serum samples is due to the presence of the red blood cells in the blood sample. The contents of the RBC will be liberated into the plasma[15].Also the constituents of the blood cell can interfere directly with the measurement of creatin kinase activity by adenylate kinase released from erythrocytes which increased the enzymatically measured of ck[16].The increased in the activity of LDH enzyme is perhaps related to the change in cell permeability, and to the fragility of erythrocyte membrane during prolonged storage ,and may reflect a decrease in ATP concentration in the erythrocytes[17]. As a result , in the analysis of any sample we should take 3 important things in account to get right results and right diagnosis. The first is what sample we are analyzing, what constituent we measure, and lastly how much was the sample delayed before the analysis is done . All these 3 factors affect the diagnosis from the result of the sample analysis, especially for the enzymes measurement where specimen should be separated within 3 hours and stored as serum ,stored at 4C°.

Tables:

Table (1)characteristics of subjects who participate in the study

characteristics	Value
Total number	51
Male	32
Female	19
Weight (Kg)	63±8.3
High (cm)	172±7.6
Serum CK (IU/L)	54.3±15.4
Serum LDH (IU/L)	251±34.6

Table (2)effect of storage , at 4 c° on the activities of CK and LDH enzymes in serum sample (N=51)

parameters	Storage time	
	0 time	24 hours
CK (IU/L)	54.3±17.1	55.7±15.4
LDH (IU/L)	251.2±34.6	253.3±32.7

Table (3)effect of storage , at 4 c° on the activities of CK and LDH enzymes in plasma in contact with Red blood cells sample (N=51)

parameters	Storage time	
	0 time	24 hours
CK (IU/L)	55.32±15.5	69.8±12.5**
LDH (IU/L)	262 ±37.6	292±40.4**

P<0.01

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تأثير الخزن على فعاليه انزيم الكرياتنين كاينيز ولاكتيت ديهايروجنيز في الدم ومصل الدم

انتصار عبد اللطيف عبد الرضا*

الملخص

لغرض فحص احتمالية خزن نموذج الدم او مصل الدم من ان يسبب تغيرا" في فعالية بعض الانزيمات، تم قياس مستوى فعالية انزيم CK و انزيم LDH في نموذج الدم ومصل الدم خلال فترة خزن لمدة ٢٤ ساعة ، تم اختيار ٥١ شخصا" (٣٢ من الذكور و ١٩ من الاناث) معدل اعمارهم ٢٠ سنة (تتراوح من ١٨ الى ٢٢ سنة)٠ كانوا من غير المدخنين والذين هم ظاهريا" اصحاء وان معدل اوزانهم ضمن الحدود الطبيعية والذين لا يعانون من مشاكل صحية ومن غير المتعاطين للادوية خلال الاسبوع الماضي من الدراسة، وقد تم قياس مستوى فعالية انزيم CK وانزيم LDH لهم في الاوقات صفر، ٢٤ ساعة ، لم يؤثر الخزن على مستوى فعالية الانزيمات وبقيت تقريبا" ثابتة. اما في نموذج من بلازما الدم فان تاثير الخزن لمدة ٢٤ ساعة قد سبب ارتفاعا" معنويا بمستوى فعالية الانزيمات عندما حفظ نموذج الدم بدرجة ٤ درجة مئوية لمدة ٢٤ ساعة. واستنادا" لهذه النتائج نوصي بضرورة فصل مصل الدم عن كريات الدم الحمراء لان بقاء كريات الدم الحمراء لمدة ٢٤ ساعة دون فصلها يؤدي الى حدوث تغير بمستوى فعالية بعض الانزيمات.

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* قسم الكيمياء الحياتية، كلية الطب، جامعة البصرة