รายงานผู้ป่วย

# Total and partial determination in biochemistry laboratory tests : Problematic cases

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*Two problematic cases in laboratory medicine about total and partial determination in biochemistry laboratory tests were discussed. The first case was 48 years old female patient with metastatic pancreatic tumor. After she had operation done, serosanguineous fluid leaked from her surgical wound. The bilirubin level in the fluid was measured. Abnormal results of bilirubin determination were found due to dilution threshold problem. The second case was 58 years old female patient presented to the physician with complaint that she felt pain in her chest. The creatine kinase determination was requested for diagnosis. In this case, abnormal results were due to principle of the test. The purpose of this report is to make awareness of the clinician in considering some basic principles of frequently requested biochemistry tests. These principles can be good explanations for possible abnormal laboratory results.*

**Key words :** *Determination, Bilirubin, Creatine kinase.*

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**วิโรจน์ ไววานิชกิจ. การวัดปริมาณองค์รวมและองค์ประกอบในทางชีวเคมีคลินิกตัวอย่างกรณีศึกษาที่เป็นปัญหา. จุฬาลงกรณ์เวชสาร 2542 ก.พ; 43(2): 109-13**

*ได้อภิปรายกรณีศึกษา 2 กรณีทางเวชศาสตร์ชันสูตรเกี่ยวกับการวัดปริมาณองค์รวมและองค์ประกอบในทางชีวเคมีคลินิก กรณีศึกษาแรกเป็นผู้ป่วยหญิงอายุ 48 ปีป่วยด้วยโรคมะเร็งทุติยภูมิของตับอ่อน ภายหลังจากการผ่าตัดพบสารคัดหลั่งคล้ายเลือดซึมออกมาทางแผลผ่าตัด ได้วัดปริมาณของ bilirubin ในสารน้ำนี้ พบความผิดปกติของผลการตรวจเนื่องจากปัญหาเกี่ยวกับระดับวิกฤตของความเจือจาง กรณีศึกษาที่สองเป็นผู้ป่วยหญิงอายุ 58 ปีป่วยมาพบแพทย์ด้วยอาการเรื่องอาการเจ็บอก ได้วัดระดับ creatine kinase เพื่อช่วยในการวินิจฉัย ในผู้ป่วยรายนี้ผลการตรวจที่ผิดปกติเกี่ยวข้องกับหลักการของการทดสอบโดยตรง วัตถุประสงค์ของบทความนี้เพื่อที่จะช่วยให้แพทย์ได้ตระหนักถึงหลักการเบื้องต้นของการตรวจทางห้องปฏิบัติการทางเคมีคลินิกที่ใช้บ่อย หลักการเหล่านี้สามารถเป็นคำอธิบายที่ดีสำหรับผลการตรวจทางห้องปฏิบัติการที่ผิดปกติซึ่งอาจเกิดขึ้นได้*

Clinical chemistry laboratory plays important role in medicine. Diagnosis and follow-up diseases must base on patient history, physical examination and laboratory results.[1] Many laboratory tests in clinical chemistry can be divided to total and partial determination.[2] The two methods are useful in differential diagnosis of diseases. Serum bilirubin level can be measure as total bilirubin and direct bilirubin. Many enzymes can be measured as total activity or isoenzyme activity. The sum quantity of compositions measured by partial determinations should be equal to the quantity measured by total determination. Sometimes there may be some errors in determination. There are many reasons for laboratory abnormality.[3] Some errors are laboratory in origin. Others are medical personnel errors. Some abnormal laboratory results are due to specific aspects in laboratory medicine. The major notification that physician should realize is that methods for total and partial determination are not same, therefore, some strange results as partial determination provide higher result than total determination can occur. Every physician should understand the basic principle of tests they request. In this article, we reported two problematic cases about total and partial determination of chemical substances due to interesting principles in laboratory medicine.

**Case 1**

A case of 48 years old female patient with underlying breast carcinoma Stage II post total mastectomy was reported. She presented to the doctor with the major problem of jaundice for about 5 months. She was diagnosed to have pancreatic metastasis tumor by fine needle aspiration. She had Whipple's operation done. After tumor resection procedure, serosanguineous fluid leaked from her surgical wound. This fluid was collected in the bag and then it was sent to the clinical chemistry laboratory to measure bilirubin level.

The fluid was analyzed by the automated biochemistry. At first, the level of total bilirubin was 1.79 mg/dl and the level of direct bilirubin was 2.05 mg/dl. Repetition analysis was done using another analyzer but the results were the same as the first time. At last, the specimen was diluted then analyzed. The level of total bilirubin was 1.75 mg/dl and the level of direct bilirubin was 1.55 mg/dl. These results were reported to the physician.

The fluid still leaked from this patient's wound for days. This patient died on day 9 after operation.

**Case 2**

A case of 58 years old female patient was reported. She complained symptom of chest pain for hours to the physician. The electrocardiogram (EKG) was within normal limit. So the physician requested for creatine kinase test to rule out myocardial infarction.

The blood specimen was analyzed for total creatine kinase (total CK) level and creatine kinase isoenzyme MB (CK-MB) by the same automated biochemistry. The level of total CK was 51 U/l and the level of CK-MB was 53 U/l. Repetition analysis was done but the result was the same. These results were reported to the physician.

This patient was not diagnosed to have myocardial infarction. From review systemic, she revealed that she had underlying lung disease for years.

**Discussion**

These two cases are examples of problematic cases in total and partial determination of chemical

substances in body fluids. Both cases revealed abnormal laboratory results. The sum quantity of compositions exceeded the total quantity. Decision in interpretation these abnormal laboratory results was very difficult.

The first case was the case that direct bilirubin level exceeded total bilirubin level. To explain this phenomenon, the principle of dilution threshold[4] should be used. Considering general chemical reaction as the following. [5]:

substrate + reagent ⟶ product

Substrate is the chemical substance that must be measured. Reagent is the chemical substance added in order to start the reaction. Product is the chemical substance that is measured to imply the level of substrate. There are two situations to be considered.

1. When reagent exceeds substrate, it is not necessary to determine the quantity of the left reagent because the quantity of reagent is determined at the first time.
2. When substrate exceeds reagent, it is necessary to determine the quantity of the left substrate because the product cannot be the representation of total substrate.

So there must be limitation of substrate or specimen concentration for every reaction. This limitation is called dilution threshold. If specimen concentration exceeds dilution threshold, there will be aberration in laboratory result.[4-7] In this case, the concentration of the serum bilirubin was so high that the laboratory results were error. (Serum total bilirubin level is measured by DPD method with dilution threshold 30 mg/dl. Serum direct bilirubin level is measured by Jendrassik method with dilution threshold 10 mg/dl.) In case that the level of chemical substance is very high, the dilution technique should be considered.

The second case was the case that CK-MB level exceeds CK level. Usually isoenzyme level should not exceed the total enzyme level.[8] There must be something error if the isoenzyme level exceeds total enzyme level. In this case, the level of CK-MB (53 U/l) exceeds the level of total CK (51 U/l). What is the reason? Total CK level is measured by optimized standard method using UV test. So the quantity of total CK by this method is implied directly by determination of product of chemical reaction. While CK-MB determination base on principle of immunoassay. A specific antibody is used to inhibit the CK-M moiety without affecting the CK-B moiety. In this method, conclusion that CK-B fraction accounts for one half the activity of CK-MB is accepted. The CK- B fraction is determined by the NAC-activated method. The CK-MB level is assumed to be equal to two times of CK-B level. Therefore, the presence of isoenzyme CK-BB may stimulate an increased proportion of CK-MB in the immunologic CK-MB assay. CK-BB can stimulate falsely elevated CK-MB exceeding 35% and even up to twice the total CK activity. CK-BB can be measured in the serum after damage to many organs such as brain, liver, kidney and lung.[9-10] And atypical CK isoenzyme called macro CK[11] can be found in 3 - 6 % of hospitalized patient especially in elderly female patient. In this situation, the total CK is usually in the normal range but high CK-MB level can be observed. In this patient, these two reasons can be good explanation for why CK-MB level exceeds total CK level. The limitation of biochemistry tests for determination of isoenzyme was discussed. Another alternative method for measure isoenzyme such as electrophoresis should be used in problematic cases.

These two cases are examples of problematic cases in clinical biochemistry. To measure one chemical substance, there are many variations that can affect laboratory results. Due to the increasing in laboratory requests, abnormal laboratory results increase. Many laboratory tests can be separated in partial and total determination. Abnormal excess of compositions than total amount in these tests seems very interesting. Many explanations can be given for each situation. To find the resolution of the problem is important. But how to give the best accurate results to the patient is more important.

## Conclusion

Two cases of abnormal biochemistry laboratory results were reported. Abnormal excesses of compositions than total amount from partial and total determination were observed. The abnormality in the first case is due to the dilution threshold problem. The second problematic case was due to the chemical reaction principle.

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