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Effects of storage and transport conditions on accuracy and precision of homocysteine analysis and concentrations

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Abstract This is a known and documented fact that several pre-analytical steps are essential and mandatory for accurate and precise analysis of biomarkers such as correct container, volume, collection, transport and storage. Our present study described the effects of storage and transport conditions on accuracy and precision of homocysteine (Hcy) analysis and concentrations. Hcy blood samples were subjected to several artefactual condition and relevant scenario to assess the deviations. Data obtained showed deviation of 14.52% to 23.0% and 34.25% to 47.75% in Hcy concentration during delays in transport, storage and analysis. Hcy is a critical care biomarker and its accurate and precise analysis is significantly important to ensure correct diagnosis and assessment of progression of treatments. Clinical laboratory thus need to safeguard the documented standards and protocols and ensure its implementation when Hcy test is requested.

Keywords Homocysteine, Accuracy, Precision
Short Title: Effects on Precision and accuracy of Homocysteine analysis

Introduction

Chronic diseases, such as neuropathy, diabetes, inherent metabolic disorders, and renal insufficiency are getting more common since start of current decade and more and more adult population seems to be suffering from either of the mentioned clinical conditions or multiple syndromes [1]. In order to diagnose the individuals, who developed such diseases and in danger of having complications, hospitals, clinics and care givers must have proper diagnostic tools to identify the actual ailment and possible treatment regiments [2,3]. Identifying or suggesting a biomarker that can provide information about etiology and/or progression of a disease is as important as its treatment. Collecting blood or fluid at time of natural history or progression of disease is done either at clinics, wards, Lab collections centers and transported and stored as per standardized procedures. Transport and storage of biological samples, from which an intended, clinically significant biomarker is needed to be analyzed, is very important for the correct, accurate results [4,5]. Homocysteine is an important biomarker of sulfur-containing amino acid synthesized in the body as an intermediate of methionine metabolism [6]. Its elevated levels in blood samples represent several pathological ailments such as neurodegenerative disease, bone dystrophy, renal and cognitive impairment and development of congenital defects [6]. Moreover it's an independent risk factor to specify cardiovascular and cerebrovascular diseases [7-9]. This is a known and documented fact that several pre-analytical steps are essential

and mandatory for accurate and precise analysis of biomarkers such as correct container, volume, collection, transport and storage.

Any deviation in mentioned steps and requirement cause inaccuracy and deviation in expected true results. Our present study described the effects of storage and transport conditions on accuracy and precision of homocysteine (Hcy) analysis and concentrations.

Materials and Methods

Thirty healthy individuals were selected for this study to avoid analytical biases and also to avoid clinical disagreements. Three types of case or untoward condition were created to check effects of transport and storage on homocysteine concentrations. For each scenario, a different day was selected to collect samples from same 10 individuals, which were dedicated for Condition 1-3. Condition 1: N = 10, where samples were subjected to three different storage and transport time intervals (Fig 1). Condition 2: N = 10, where samples subjected to three different time interval-delays in Transport without ice (Fig 2). Condition 3: N = 10, sample were subjected to three different time interval-delays in analysis (Fig 3). In each condition, ten samples each form same individuals were further sub grouped as per scenarios for various transport, storage and delays in analyses condition-entitling them as Scenario 1,2, 3 within each main conditions 1-3. Details are described in results and in Figures as well. Homocysteine was analyzed as per protocol provided by Roche Diagnostic (Basil). To have a variable array of data and results, individual samples were plotted as per condition (X axis), against obtained homocysteine value (Y-axis). Condition were detailed in title of the figures whereas case scenarios were depicted in series legends. Individuals ensured to abide by routine diet and avoided consumption of Vitamins B12 and folic acid during the course of present study.

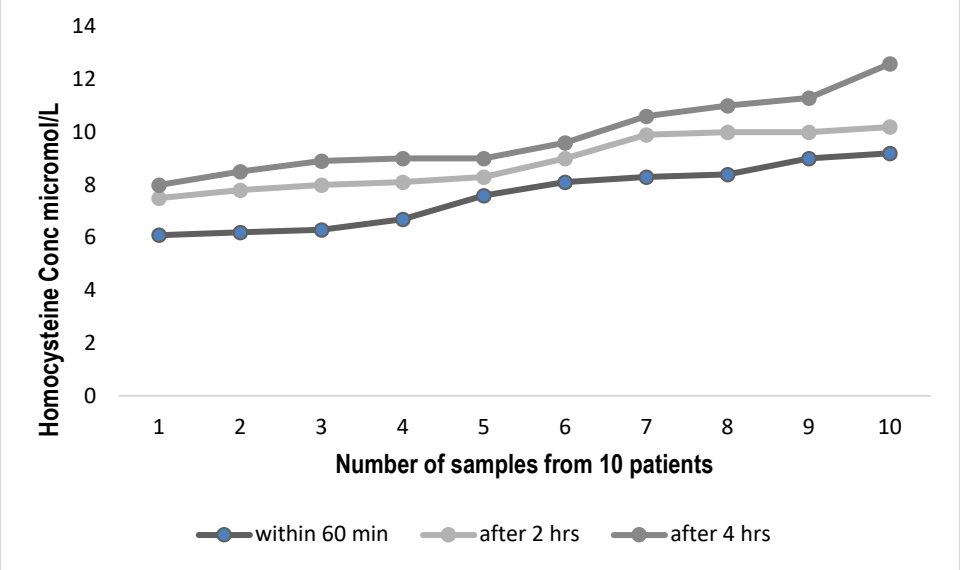

Figure 1: Homocysteine levels after subjected to 3 storage and analysis time intervals (study 1, 2, 3) at Room temperature

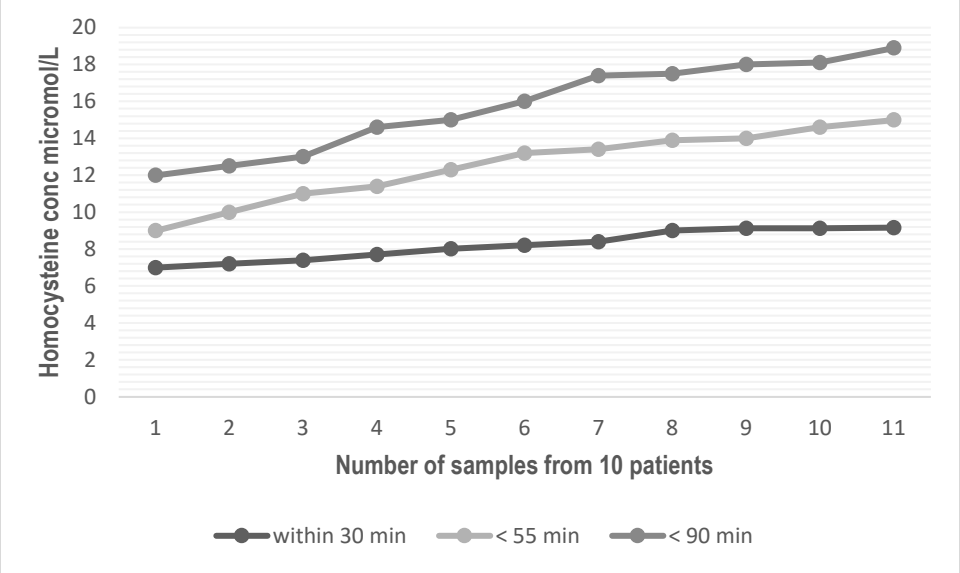

Figure 2: Homocysteine levels after subjected to 3 different time interval delays in Transport without ice

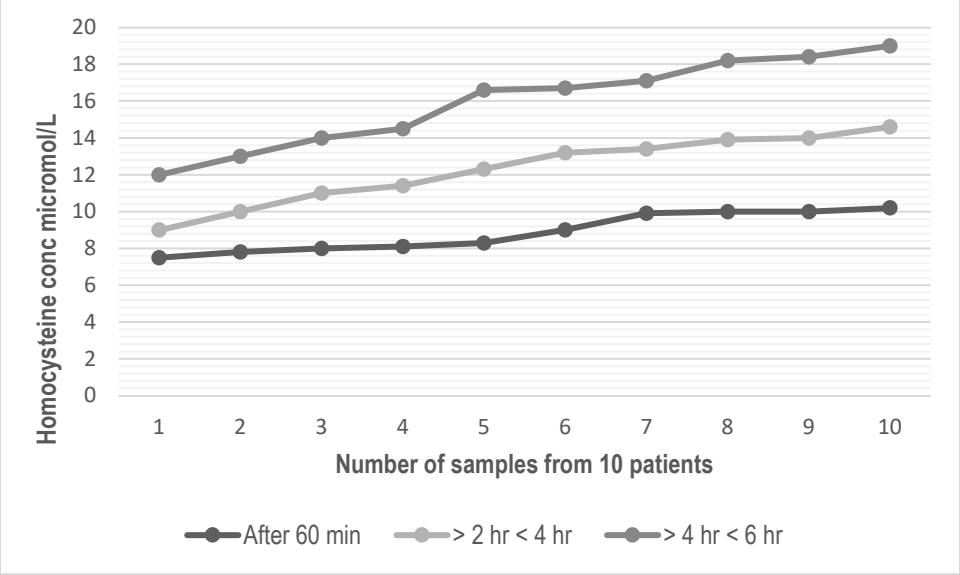

Figure 3: Homocysteine levels after subjected to 3 different time interval delays in analysis

Results and Discussion

Results are summarized in Fig1 to 3. Routine protocol for transport and analysis of homocysteine is sample collection in EDTA bottle, transport on ice within 30 minutes to lab and analysis ASAP. For current study thirty individual samples from same number of healthy, aged 25-56 years individuals, either gender, were collected, grouped as N = 10 in three main conditions of; 1: where samples were subjected to three different storage and transport time intervals (Fig 1), condition 2: where samples subjected to three different time interval-delays in Transport without ice (Fig 2) and Condition 3: sample were subjected to three different time interval-delays in analysis (Fig 3). All three condition were sub grouped three further scenario each and depicted with specific legends. Different days were dedicated for each scenario. Condition 1 (Fig 1): scenario 1 = Normal, received within 30 minutes EDTA ice, analyzed within 60 minutes, scenario 2 = received within 30 minutes EDTA ice, analyzed after 2 hrs storage at room Temp of $22^{O}C$, scenario 3 = received within 30 minutes EDTA ice, analyzed after 4 hrs storage at room temp $22^{O}C$. In this study, samples were subjected to long hrs storage at room temperature, which is not

advisable, although sample collected, transported properly within time limit. Except scenario 1, where Hcy analyzed within 60 minutes after receiving, scenario2 and 3 showed increased in homocysteine level, when subjected to long time storage of 2 hrs and 4 hrs. Condition 2 (Fig 2): scenario 1 = Normal treatment, samples received within 30 minutes EDTA ice and analyzed within 60 minutes, scenario 2 = Sample collected but transported without ice after 40 minutes but less than 55 minutes, scenario 3 = sample collected but transported without ice after 70 minutes but less than 90 min. In this condition and scenario 2 and 3, samples collected but not only transported without ice from ward to lab which is deviation from policy but delayed in transport, for 40 minutes and 70 minutes. Condition 3 (Fig 3), scenario 1 = sample received within 60 minutes, EDTA on Ice , analyzed after 60 minutes of storage at temp $26^{O}C$; scenario 2 = sample received within 60 minutes on ice analyzed after 2 hrs., but less than 4 hrs., while storing at $26^{O}C$; scenario 3 = sample received within 60 minutes on ice, analyzed after 4 hrs, but less than 6 hr., while storing at $26^{O}C$. Comparative analysis done amongst scenarios showed variable deviation in obtained results such that when Condition 1: scenario 1 (S1) compared with scenario 2 (S2), it was noted that Hcy results are 14.52% higher in S2. Similarly when S1 was compared with S3 Hcy results are 23% higher than S1, which is highly unacceptable. Normal range of Hcy is 5.0 to 12.00 micromol/L and falsified changes of even 1 increment below or above is detrimental for diagnosis of CVS or CNS diseases. Moreover, delay or storage at room temperature, even as low as $22^{O}C$ caused artefactual increase in Hcy concentration. Similarly Condition 2: S 1 vs S2 was noted to be 34.25% higher and S1 vs S3 even higher viz 47.75% when sample was transported without ice and additionally delayed in analysis.

As stated earlier elevated levels of Hcy depicts several pathological ailments such as neurodegenerative disease, bone dystrophy, renal and cognitive impairment and development of congenital defects [6]. Furthermore, Hcy is reported to be an independent risk factor to identify cardiovascular and cerebrovascular diseases [7-9]. More recently, Hcy levels gain importance in pregnancy to determine heightened risk of adverse pregnancy and untoward outcomes [6]. Few such outcomes were reported to be small size for gestational age at birth, preeclampsia, recurrent abortions, low birth weight, intrauterine growth restriction, and neural tube defects. In recent years, clinicians and cardiologist also focused on clinical utility of Hcy in cardiovascular diseases in children and adolescents. Main focus was to examine postural tachycardia syndrome (PoTS), congenital anomalies (congenital adrenal hyperplasia) and heart failure. As reported PoTS is a common functional cardiovascular disease in children and adolescents, thus having Hcy as an indicator became an important entity for the same. Reviewing the data that we have studied and the experiment that we have performed, it's obvious that deviation from collection, transport and delays in storage and analysis can caused artefactual elevation in Hcy concentration, which is disadvantageous to reach a critical clinical decision.

Conclusion

Our present study demonstrated the effects of storage, transport conditions and delays on accuracy and precision of homocysteine (Hcy) analysis and concentrations. Hcy blood samples were subjected to several artefactual condition and relevant scenario to assess the deviations. Data obtained showed deviation of 14.52% to 23.0% and 34.25% to 47.75% in Hcy concentration during delays in transport, storage and analysis. Hcy is a critical care biomarker and its accurate and precise analysis is significantly important to ensure correct diagnosis and assessment of progression of treatments. Clinical laboratory thus need to safeguard the documented standards and protocols and ensure its implementation when Hcy test is requested.

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