SHORT COMMUNICATION

EFFECT OF RECENTRIFUGATION ON THE LEVELS OF HIGH SENSITIVITY TROPONIN I

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ABSTRACT

Objective: To compare the levels of high sensitivity troponin I in plasma after specimen re-centrifugation in patients presenting with acute coronary syndrome.

Study Design: Cross sectional study.

Place and Duration of study: Department of Pathology, Armed Forces Institute of Cardiology, Rawalpindi, from Sep to Nov 2017.

Material and Methods: Seventy five patients' plasma samples of high sensitivity troponin I levels, exceeding the value of 0.06ng/ml were analyzed. Blood was collected in K/EDTA evacuated tubes and plasma was separated after an initial centrifugation (2000 g, 10 min; not refrigerated). The samples were recentrifuged after first analysis. High sensitivity troponin I was measured through the chemiluminescence technique on ADVIA CENTAUR XP immunoassay analyzer. Normally distributed continuous variables were presented as Mean ± SD and the others as median. Two-tailed Wilcoxon analysis was applied to determine the difference in high sensitivity troponin I specimens before and after recentrifugation. A *p*-value <0.05 was considered as statistically significant.

Results: Median value of high sensitivity troponin I was 4.051ng/ml before re-centrifugation and 3.689 ng/ml after recentrifugation. About 2.5th to 97.5th percentile interval was 0.020-47.23 and 0.054-48.7 ng/ml, before and after recentrifugation, respectively. Difference between the two samples were statistically analyzed by using two-tailed Wilcoxon analysis that showed a statistically significant difference (*p*-value<0.01).

Conclusion: Recentrifugation of specimens is followed by a significant reduction in high sensitivity troponin I levels which may result in misdiagnosis and treatment of the patients.

Keywords: High sensitivity troponin I, Recentrifugation.

INTRODUCTION

Cardiac troponins (cTn) have a major role in the diagnosis of myocardial infarction. Analytical performance of these assays is thus critical in diagnosis of patients with acute coronary syndrome (ACS)[1]. International consensus guidelines define acute myocardial infarction (AMI) in terms of rise and fall of cardiac troponins[2]. However since the time troponins have been introduced, problem of false positive results arose. Particulate matter such as fibrin strands were thought to be responsible for such results, therefore many researchers suggested the process of recentrifugation of the samples with high results[3].

Various pre-analytical factors such as specimen type, hemolysis, lipemia, icterus, micro-clots and debris have been reported to affect the results of cardiac troponins[1]. Lipids and proteins have been reported to interfere with troponin assay[4]. Relative centrifugation force (RCF) has been studied to see its effect on these interferants revealing that low RCF of >300 for 30 min does have an effect on high sensitivity troponin assay. However recentrifugation was recommended for pooled patient based quality controls or samples that have been stored for some time[5].

Researchers further argued that if recentrifugation is carried out for all specimens it may also result in false negative results which increases the chance of missing patients with

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ACS[6]. High speed centrifugation can cause adherence of troponins to the walls of the test tube resulting in lower values. A study carried out by Canovi et al in 2015 compared the results of conventional troponin in plasma before and after recentrifugation, concluding a significant reduction in the levels of the troponin[7].

However the question for high sensitivity troponin regarding this problem still remains unanswered. This study was planned to see the effect of recentrifugation on high sensitivity troponin assay in plasma samples of patients presenting to the Armed Forces Institute of Cardiology, Rawalpindi with symptoms of acute coronary syndrome.

MATERIAL AND METHODS

It was a cross sectional study carried out in the department of Pathology, Armed Forces Institute of Cardiology, Rawalpindi, from Sep to Nov 2017. Seventy five samples of patients that reported to emergency reception of hospital with symptoms of acute coronary syndrome were analyzed. Blood was collected in K/EDTA evacuated tubes. Specimens revealing obvious hemolysis, lipemia, and icterus were excluded. Specimens stored in refrigerator were also excluded from the study considering stability of the specimen also affects results of the assay. High sensitivity troponin I was measured through the chemiluminescence technique on ADVIA CENTAUR XP immunoassay analyzer using Trop I Ultra (SIEMENS ADVIA CENTAUR). Performance of the assay was monitored using two levels of quality control material. Analytical CV of the assay was <10%. Centrifuges were calibrated by biomedical engineers and verification of calibration was done by laboratory technologists. Samples which had higher troponin levels exceeding the positive cutoff of >0.06 ng/ml were separated and stored at room temperature in plastic tubes and were recentrifuged (2000 g, 10 min) followed by reanalysis of troponin levels.

Data Analysis

Troponin I and age were represented as median. Two-tailed Wilcoxon analysis was applied to determine the difference in high sensitivity troponin I specimens before and after recentrifugation. Bland Altman plot was constructed to see the difference betweeen the two samples. Passing- Bablock regression was done to quantify the difference. A *p*-value <0.05 was considered statistically significant.

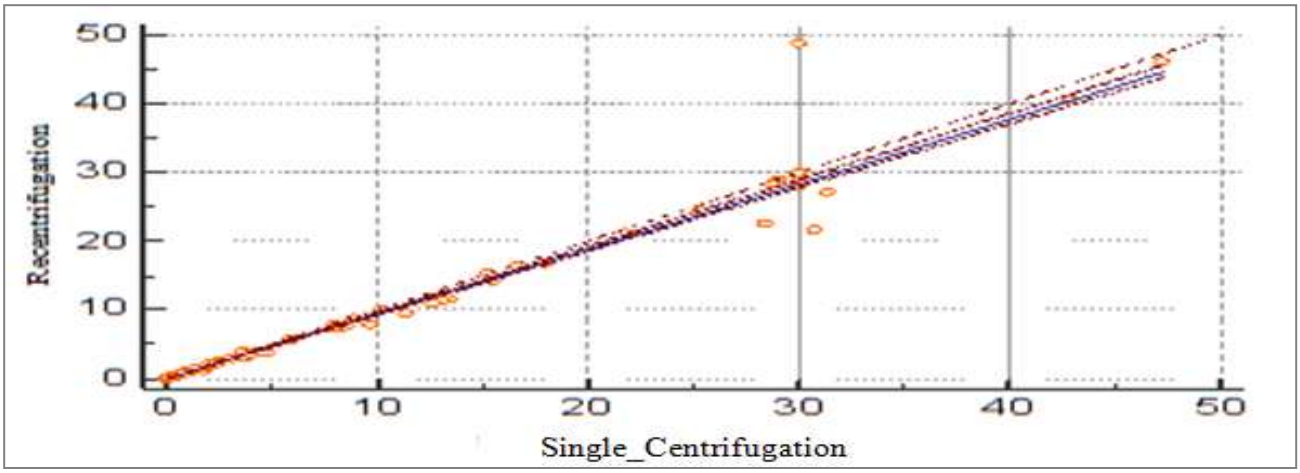

Figure-1: Passing Bablock regression. Dashed line indicates identity line; bold line, regression line. Intercept: 0.0174ng/ml (95% confidence interval [CI], -0.061 - -0.0031 ng/ml), slope: 0.943 (95% confidence interval [CI], 0.928-0.965).

RESULTS

A total of 75 patients' serum samples were collected on consecutive days. Normality of data was checked by Shapiro Wilk test that showed

p-value <0.01, showing that the data was non-Gaussian. Median age of the patients was 59 years with IQR of 17 years. Median value of high sensitivity troponin I before and after recentrifugation was 4.051 ng/mL and 3.689 ng/mL, respectively, whereas 2.5th to 97.5th percentile interval was 0.020 ng/mL to 47.230 ng/mL (95% CI,1.731-9.337) and 0.054 ng/mL to 48.7 ng/mL (95% CI, 1.436-8.291), respectively.

To see whether the difference between the two samples were statistically significant, two tailed Wilcoxon analysis was carried out that showed a *p*-value<0.01, showing that the samples after recentrifugation had a significantly lower value of high sensitivity troponin I. In order to see the effects of recentrifugation on results near the cut off, we analyzed the data by using non parametric Passing- Bablock regression which showed a median reduction of 0.0174 ng/ml (95% CI,-0.061–0.003 ng/mL) after recen-trifugation of troponin I concentration that was statistically significant (*p*-value<0.01) (fig-1). A Bland Altman plot for the data was constructed to see the difference between the two samples showing mean of 8.4 ng/ml with SD of ± 1.96 ng/ml (fig-2).

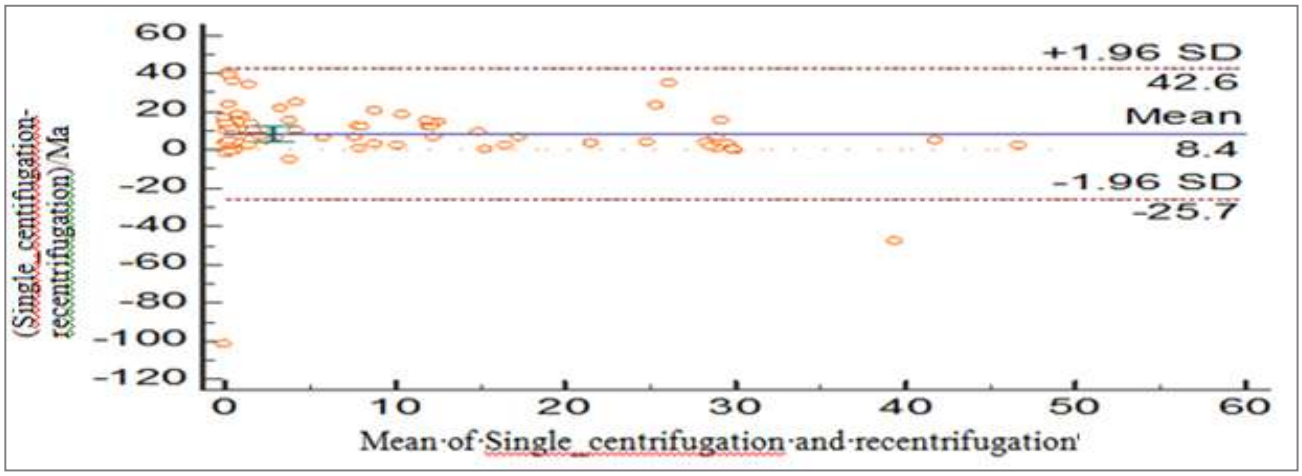

Figure-2: Bland Altman plot showing a difference between the two samples, blue line shows the mean difference showing a mean value of 8.4 ng/ml with SD of ± 1.96 ng/ml.

DISCUSSION

Cardiac troponins have been studied for possible interferences in various studies. This study was carried out to see the effect of recentrifugation that was previously observed by researchers while dealing with high troponin levels[8]. Various pre-analytical, analytical and post-analytical factors have been reported to affect the results of cardiac troponins I, but we focused only on recentrifugation.

Effect of recentrifugation on the conventional troponin levels has previously showed a negative bias of 0.01 ng/ml in a previous study[3], but it did not include high sensitivity troponin I. Limit of detection of this assay was 0.017 ng/mL and the limit of quantification was 2.05 ng/mL. Data was checked for any outliers to further ensure the reliability of the results since the effect of outliers on troponin assay has been reported earlier[9]. Importance of quality control has been emphasized repeatedly for such assays, so it was assessed by using two levels of control materials[10].

In this study, recentrifugation resulted in reduction in the levels of high sensitivity troponin I to 0.017 ng/mL (95% CI, -0.060 to -0.003) which was statistically significant (*p*-value <0.01), this finding being consistent with the study mentioned previously[7]. Reason for this negative bias however remains a question. Some researchers think it could either be due to degradation of cardiac troponin or its adherence to the walls of the tube[6]. Whatever the reason may be, recentrifugation may result in

misdiagnosis and subsequent wrong treatment of the patient. The process of unnecessary recentrifugation may also increase the turnaround time of the assay, thus resulting in delay in management of the patient.

CONCLUSION

Recentrifugation of specimens is followed by a significant reduction in high sensitivity troponin I levels which may result in misdiagnosis and treatment of the patients.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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