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Research Article

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Diagnostic efficacy and comparative precision analysis of Adenosine Deaminase (ADA) in pleural fluids from tuberculosis patients on semi-automated and fully automated chemistry analyzers

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Abstract *Background*: ADA is its usage in the diagnosis of tuberculosis and/or tuberculous pleural effusions. Comparative analytical assessments of various techniques and/or instruments and regression comparison are some of the analytical protocols used to check precision and accuracy of in-vitro diagnostic (IVD) methods. *Aim*: The present study described the comparative precision analysis of ADA by semi-automatic method (Randox Monza) and by fully automated analyzer (Cobas c311). *Materials and Methods*: Seventy five patients (males = 56; females = 19, age range = 25-76 yrs) with pulmonary congestion, chronic cough or with known history of Tuberculosis, and/or imaging confirmation of Tuberculous mass were included in this prospective study by classifying in four groups I-IV with group I being the control group of healthy individuals. ADA was estimated by Diazyme kit as per manufacturer advice in Pleural samples on Cobas c311 using Dry chemistry technology (Roche Diagnostics, Basil) and Randox Monza (Randox, UK) using enzymatic colorimetric method with reference range 6.8-18.2 U/L. The data was compared statistically by using SPSS ver 18.0 (USA), regression correlation analysis and considered significant when $P < 0.05$. *Results*: Analysis of samples data depicted compatible and appreciable precision amongst both instruments with regression R^2 ranging from 0.964 to 0.989, representing attuned accuracy of 97.6% to 98.9% for pleural samples of TB patients. Conclusion: It is concluded that usage of either semi automated or fully automated instruments for determination of ADA with variable numerical of efficacy, will still produce compatible precision of more than 90%, advocating its stability and sustainability of diagnostic utility.

Keywords Diagnostic efficacy, Adenosine Deaminase, tuberculosis patients

Introduction

Adenosine Deaminase (ADA) is an important enzyme, responsible for purine metabolism [1,2]. It facilitates the catalysis reaction of hydrolysis and deamination of adenosine to inosine deoxyadenosine to decoxyinosine [1,3]. One of the most important and clinically significant utility of ADA is its usage in the diagnosis of tuberculosis and/or tuberculous pleural effusions [1,2,4]. Variable sensitivity and specificity has been reported for ADA, with assurances of its merit, non-invasiveness and been comparatively inexpensive [1,5,6].

Tuberculosis, been recognized as the second deadliest infection after HIV, always remained at the center of immense attention from WHO, clinicians, infectious diseases experts and pulmonologists [1,2,4] regarding timely identification and sensitivity of diagnostic methods and other investigative techniques [7,8].

Comparative analytical assessments of various techniques and/or instruments and regression comparison are some of the analytical protocols used to check precision and accuracy of in-vitro diagnostic (IVD) methods. The present study described the comparative precision analysis of ADA by semi-automatic method (Randox Monza) and by fully automated analyzer (Cobas c311).

Materials and Methods:

Selection of patients and healthy controls

Seventy five patients (males = 56; females = 19, age range = 25-76 yrs) with pulmonary congestion, chronic cough or with known history of Tuberculosis, and/or imaging confirmation of Tuberculosis mass were included in this prospective study from January 2018 to November 2018. The patients, who are on drug therapy, underwent surgery, suffering from cardiac or renal impairment was excluded from the study. Twenty five samples were also taken from Age-gender matched individuals with no history of any adverse clinical condition.

Estimation of Adenosine Deaminase in plasma samples

ADA was estimated by Diazyme kit as per manufacturer advice. Briefly, method was base of enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by an enzyme purine nucleoside phoshorylase. Addition of Xanthine oxidase coverts Hypoxanthine to uric acid and H2O2, which further forms a quinone dye after addition of N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase. Intensity of color of quinine dye is directly proportional to the concentration of ADA is either plasma or pleural samples.

Analytical Measurement of ADA on semi-automated Randox Monza and automated chemistry analyzer Cobas c311

Pleural samples were collected from 75 patients and 25 healthy individuals (Fig 1) in heparinized tubes. The patients were divided into three groups with 25 patients in each group, according to severity of pulmonary conditions, Group II includes suspected cases of pulmonary congestions, fever and chronic coughs, group III includes imaging confirmed cases of Tuberculosis with plasma ADA positive results, and Group IV includes confirmed cases of Tuberculosis, with imaging and plasma, pleural fluid positive ADA. Pleural samples were centrifuged and analyzed for ADA on Cobas c311 using Dry chemistry technology (Roche Diagnostics, Basil) and Randox Monza (Randox, UK) using enzymatic colorimetric method with reference range 6.8-18.2 U/L. The data was compared statistically by using SPSS ver 18.0 (USA), regression correlation analysis and considered significant when $P < 0.05$.

Results

Results are summarized in Fig 1 to 4. The patients were divided into three groups with 25 patients in each group, Group II includes suspected cases of pulmonary congestions, fever and chronic coughs (Fig 2), group III includes imaging confirmed cases of Tuberculosis with pleural ADA positive results (Fig 3) and Group IV includes confirmed cases of Tuberculosis, with imaging and plasma, pleural fluid positive ADA (Fig 4). Samples were analyzed for ADA on Cobas c311 using dry chemistry technology (Roche Diagnostics, Basil) and Randox Monza using enzymatic colorimetric technology. Data were compared using SPSS ver 18 with regression correlation equations. Analysis of samples data depicted compatible and appreciable precision amongst both instruments with regression R^2 ranging from 0.964 (Fig 1) to 0.989 (Fig 3), representing attuned accuracy of 97.6% (Fig 2), 98.9% (Fig 3) and 98.4% (Fig 4) for replicated run on two different instruments, in suspected, imagining confirmed, and added pleural fluid ADA positive cases. Regression analysis showed y equation as $y = 0.660x-2.895$ (Fig 1), $y = 0.921x+1.086$ (Fig 2), $y = 0.994x+0.294$ (Fig 3) and $y = 1.031x-0.417$ (Fig 4).

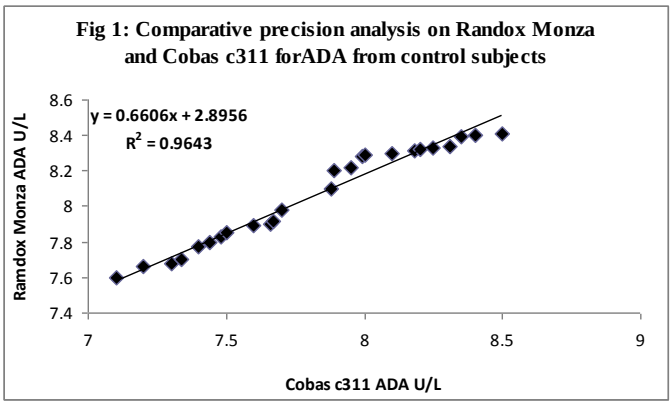


Fig 1: Comparative precision analysis on Randox Monza and Cobas c311 forADA from control subjects

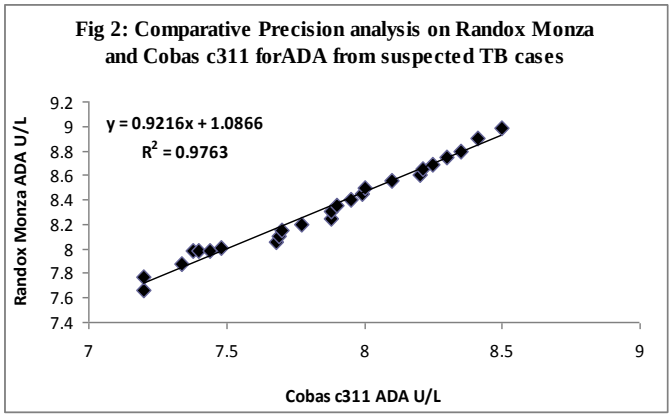


Fig 2: Comparative Precision analysis on Randox Monza and Cobas c311 forADA from suspected TB cases

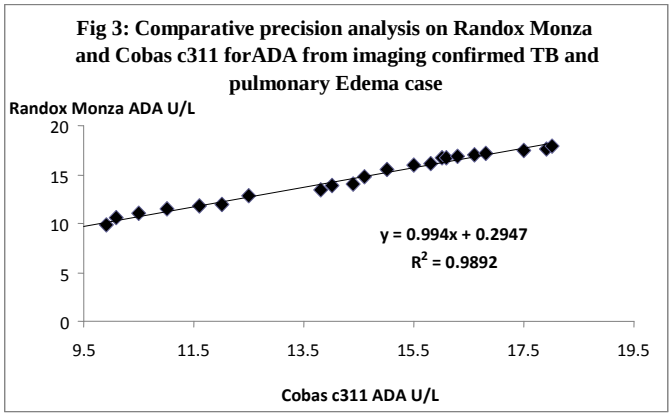
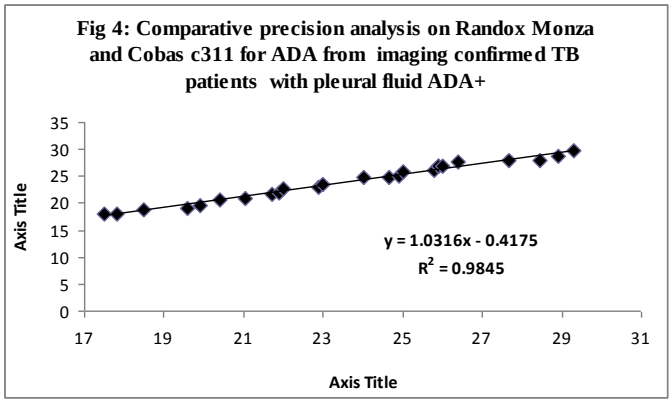


Fig 3: Comparative precision analysis on Randox Monza and Cobas c311 forADA from imaging confirmed TB and pulmonary Edema case



Discussion

Comparative regression analysis instruments and precision evaluation is one of the main analytical protocols that can predict accuracy of in-vitro diagnostic (IVD) methods. The present study described samples from Tuberculosis patients used for comparative precision analysis of ADA through regression correlation analysis using semi-automatic method (Randox Monza) and by fully automated analyzer (Cobas c311). As stated previously, Tuberculosis is recognized as the second deadliest infection and remained at the center of immense attention from infectious diseases, Intensivist experts and pulmonologists [1,2,4] regarding timely identification and sensitivity of diagnostic methods and other investigative techniques [7,8].

ADA has long been considered as a valuable marker for diagnosis of pleural effusion in TB patients [9]. However, ADA can also be found elevated in some other proliferative diseases, thus making it highly sensitive but low in specificity [1]. Several studies conducted regarding comparison and diagnostic specificity of ADA in pleural effusion, sputum and sera of TB patients [1,10]. Although they had found variable results of ADA from different diseased groups and categories of samples, but in all cases, ADA was noted to be above the normal reference values. Concomitantly, high values, above the upper limits of 18.2 U/L correlated with the presence of disease in 93.3% cases, resulting in positive predictive value of 62% [1]. Recent and past studies also advocated the diagnostic efficacy of ADA in pulmonary tuberculosis that focused mainly on laboratory assessment ADA, in addition to specificity and sensitivity [2,4,11]. A recent study reported sensitivity and specificity of ADA as 78% and 76%, respectively, after assessing ADA in both sputum and serum samples. All studies reported showed usage of ELISA, semi automated and fully automated instruments for determination of ADA with variable numerical of efficacy, specificity and sensitivity with more than 60% and even 70% of positivity, which showed diagnostic utility of the same.

Conclusion

Present study depicted analytical precision of ADA determination in pleural fluids from TB patients on two different instruments, one been semi-automated and other fully automated chemistry analyzer. Percent accuracy of more than 90% in al groups advocated stability of methods and sustainability of diagnostic utility.

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