VOLUME - 13, ISSUE - 08, AUGUST - 2024 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Biochemistry

COMPARATIVE ANALYSIS OF RENAL PARAMETERS TEST RESULTS: FULLY AUTOMATED VS SEMI AUTOMATED BIOCHEMICAL ANALYZER

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ABSTRACT

Background: Clinical Biochemistry tests comprise over one third of all hospital laboratory investigations in India. Thus it's important to ensure overall quality management of the laboratory in terms of accuracy, reliability and timeline of reported test results. The present study aims to evaluate the test performance and the reliability of the results obtained by semi-auto analyzer as in comparison to fully-auto analyzer. **Methods:** Total 70 patients were enrolled in this study who were advised for renal function test investigation in S.N. Medical College, Agra. The blood samples were analyzed for three biochemical parameters- Creatinine, Urea and Uric Acid on both ERBA Chem 7 (semi-auto analyzer) and Selectra Pro M (fully-auto analyzer). Statistical analysis was carried out using independent student t test, Pearson correlation, Bias percentage and Bland-Altman analysis using Microsoft Excel 2016 and Python programming language. The p-value <0.05 was considered to be statistically significant. **Results:** In our study, we found a non-significant difference in the mean value of all the three parameters- urea, creatinine and uric acid between the readings obtained on fully and semi auto analyzers while the correlative study showed significant positive correlation for all the parameters. Bias percentage were all in acceptable range except the urea and the Bland-Altman analyses showed that the readings from both semi and fully auto analyzers were in agreement with each other. **Conclusion:** The findings of this study suggest that the semi-auto analyzer can be used as a reliable alternative of fully-auto analyzer.

KEYWORDS : Fully-auto analyzer, Semi-auto analyzer, Creatinine, Urea, Uric Acid.

INTRODUCTION:

In today's era clinical biochemistry labs play a vital role in diagnosing, monitoring and managing various medical conditions. In clinical biochemical lab, the analytes in the body fluids like blood, serum, plasma and urine are measured[1]. For this, systematic monitoring and evaluation of various aspects of laboratory operations are very mandatory. Quality assurance is an important key to ensure the reliability of any test results which is essential for guiding clinical decisions and ensuring optimal patient care[2]. Reliability of any test depends mainly on precision (the closeness with which repeat analyses of the same material can be made) and accuracy (it refers to the closeness to the true value) of any test performance[3,4].

There are various methods in clinical lab for analysis of sample among which classical ones include colorimeter, UV visible spectrophotometer and advanced forms include semi-automatic analyzer and fully automatic analyzer. Colorimeter and UV Visible Spectrophotometer being basic techniques includes a lot of manual work which tests the handling of the analyst i.e. pipetting, incubation, taking time lapse observation and finally the calculation part which can prove to be time consuming and difficult to use. In contrast the semi auto analyzer means that the analysis process is partially dependent on an analyst (pipetting of reagent and sample, mixing, incubation and result recording). Semi auto analyzers are advantageous with respect to cost, size and structures. But due to heavy work load fully automated analyzers are used because it does all the steps itself, the analyst only has to make sure that the machine is calibrated, cleaned and have sufficient amount of reagents[5].

However in India, many primary health care centers, small scale labs where there is not much access to advanced test techniques or during the breakdown of fully auto analyzer, the quality check of available cost efficient semi-auto analyzer is must, to assure that there is no significant variation among the results of fully and semi-auto analyzer, and they can be used as the efficient alternative of each other[6].

Therefore, the aim of our study is to compare the results of the biochemical tests performed on the semi and fully automated analyzer.

MATERIAL AND METHODS:

The present study has been carried out in the Department of Biochemistry, S.N. Medical College, Agra. Total 70 patients from OPD of medicine were enrolled in this study. 4ml of venous blood sample was collected in plain vial from all subjects under aseptic conditions with their permission. The collected blood samples were then incubated for 45 min at room temperature and were centrifuged at 3000rpm for 10-15 minutes to obtain serum. This obtained serum was analyzed for three biochemical parameters-urea, creatinine and uric acid on both ERBA Chem 7 semi-auto analyzer and ELITech Group's Selectra Pro M fully-auto analyzer by using the standardized kits.

The Internal Quality Control (IQC) has been conducted on both the analyzers. This approach includes regular calibration, performance checks and comparison of results to established standards. IQC is done to minimize the errors, to ensure the precision and accuracy of the machine and to check the reliability of the patient results.

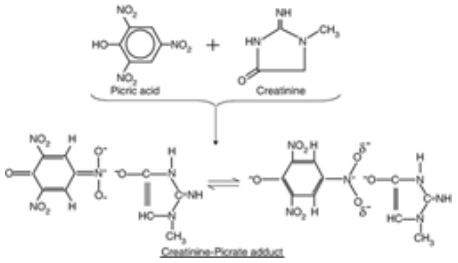
Biochemical Test's on ERBA Chem7:

The ERBA Kits are used for the following biochemical parameters.

Creatinine: The creatinine kit employing the Jaffe-Kinetics method, was used to estimate serum creatinine levels. This method is based on the principle that the rate of formation of a colored complex between creatinine and alkaline picrate is measured at 505nm. The effect of interfering substances is reduced using the kinetic procedure. This is done at Fixed Time mode (assessing the difference between an initial and final value during a specified time interval) at time intervals of

20 seconds (T1) and 60 seconds (T2).

Reaction Involved:



Urea: The Urea Kit, employing the GLDH- Urease method, was used to estimate serum urea levels. This method is based on the principle that the rate of decrease in absorbance is monitored at 340nm and is directly proportional to urea concentration in the sample. This is also done at fixed time mode at time intervals of 20 seconds (T1) and 60 seconds (T2).

Reaction Involved:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{-KG} + NADH \xrightarrow{\text{GLDH}} \text{Glutamate} + NAD$$

-KG: α-Ketoglutarate
GLDH: Glutamate Dehydrogenase

Uric Acid: The Uric Acid Kit, employing Modified Trinder Peroxidase using TBHB method, was used to estimate serum uric acid levels. This method is based on the principle that the intensity of the chromogen (Quinoneimine) formed is proportional to the uric acid concentration in the sample when measured at 505nm. This is also done at End time mode (The point where the reaction has just finished and no further changes will occur).

Reaction Involved:

$$\text{Uric Acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{TBHB} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + H_2O$$

4-AAP: 4-Aminoantipyrine
TBHB: 2,4,6-Tribromo-3-hydroxy benzoic acid

Biochemical Test's on SELECTRA PRO M:
The Q-line kits are used for the estimation of serum creatinine, urea and uric acid levels. The creatinine and urea kits follows the same method and principles as the ERBA kit while there is a difference in method of the uric acid kit as shown below.

Uric Acid: The Uric Acid Kit, employing Uricase Enzymatic method, was used to estimate serum uric acid levels. This method is based on the principle that the intensity of the chromogen (Quinoneimine) formed is proportional to the uric acid concentration in the sample when measured at 505nm. This is also done at End time mode.

Reaction Involved:

$$\text{Uric Acid} + O_2 + 2H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{4-AAP} + \text{EHSPT} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

4-AAP: 4-Aminoantipyrine
EHSPT: N-Ethyl-N-(2-Hydroxy-3-Sulfopropyl) m-Toluidine

Statistical Analysis:
All the variables is presented as mean ± standard deviation for both semi and fully-auto analyzer, calculated using Microsoft Excel 2016.

The Python programming language was used to calculate the Independent t-Test and the Pearson Correlation. The t-Test was performed to compare the means of two groups. It is used to determine if there is any significant difference between means of the two groups or not and how are they related. The p-Value is less than 0.05 was considered as statistically significant. While the Pearson correlation coefficient is calculated to check the linear correlation between the two sets of data. Significant high values of Pearson correlation coefficient ($0<PCC<+1$) shows that two sets of data have positive correlation with each other.

Bias percentage were calculated to see the variation obtained between the readings of two methods falls within the allowable range or not. This was done on the Microsoft Excel using the formula: Test Method (Mean)-Comparative method (Mean)/Comparative method (Mean) X 100 where we have taken semi-auto analyzers as test method and fully-auto analyzer as comparative method. The bias percentage were then compared to the standard allowable bias according to the European recommendations adopted by Baadenhuijsen et al., 2000[7].

Bland-Altman plot were used to evaluate the agreement among the two measurements techniques. The graph consists of two Limits of agreements (LOA) from the mean bias (calculated as the average of the difference between the values of fully and semi-auto analyzers). The two limits of agreements were calculated as Bias-1.96 X SD of difference between the two values (Lower LOA) and Bias+1.96 X SD (Upper LOA) [8]. The graph was then plotted taking X-axis as average and Y-axis as difference of the two readings individually. All the calculations were done using Microsoft Excel and the graphs were then analyzed in accordance with distribution of the readings above and below the bias level within the limits of agreements.

RESULTS:
This study was conducted on 70 patients and the results show that there is no significant difference (p-value > 0.05) among the means values of creatinine, urea and uric acid obtained from the fully and semi-automatic analyzers as shown in Table 1.

In this study, Bias % was acceptable for uric acid and creatinine while for urea a significant difference was seen between the allowable bias (6.0) and calculated bias (13.04). (Table2)

Further, correlation analysis showed a significant positive correlation (r value) between the readings of all the three biochemical parameters tested on semi & fully auto analyzers. (Table 3) (Fig1)

Table 4 shows the Bland Altman analysis, the mean difference values suggest a negligible difference in the readings between semi-auto and fully-auto analyzers. The Bland-Altman plot (Fig 2) shows that the scatter is evenly distributed along the line of no difference for all the three parameters indicating that all the readings are in agreements with each other.

Table 1 : Descriptive statistics represented by Mean ± SD, t-Test and p-Value between fully and semi auto analyzer

Parameters	Fully Auto Mean ± SD	Semi Auto Mean ± SD	t-Test	p-Value
Creatinine	1.26 ± 0.79	1.31 ± 0.65	-0.4003	0.6895
Urea	31.81 ± 25.35	35.96 ± 28.26	-0.8878	0.3763
Uric Acid	4.94 ± 2.17	5.09 ± 2.68	-0.3413	0.7334

Table 2: Comparison of two measurements methods using Bias %

Parameters	Bias %	Allowable Bias%	Result
Creatinine	4.01	4.4	Accepted
Urea	13.04	6	Not Accepted

Uric Acid	2.94	5.3	Accepted

*Standard allowable bias is according to the European recommendations adopted by Baadenhuijsen et al., 2000[7]

Table 3: Pearson Correlation between fully and semi auto analyzers

Parameters	r-value
Creatinine	0.8896
Urea	0.9169
Uric Acid	0.9178

Table 4: Bland-Altman analysis of agreement between two measurements methods

Parameters	Mean Difference Between	SD	Limits of agreement (LOA)	
	fully and semi auto analyzer		Upper	Lower
Creatinine	-0.05	0.366	0.668	-0.768
Urea	-4.149	11.289	17.977	-26.276
Uric Acid	-0.145	1.103	2.018	-2.308

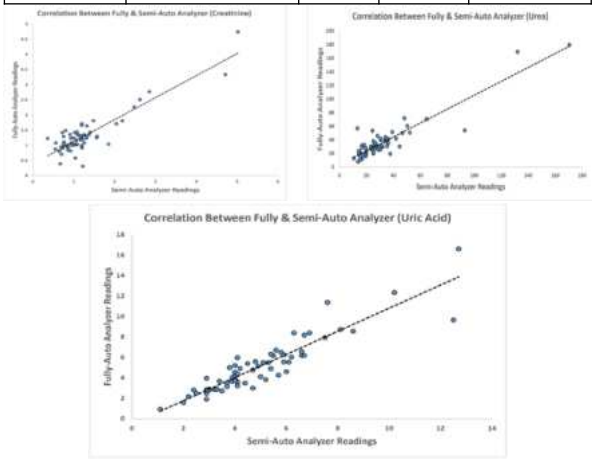


Fig1: Correlation between two measurements methods.

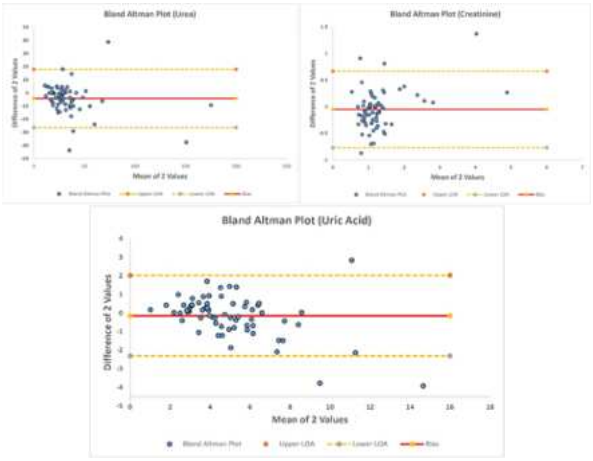


Fig2: Agreement between semi-auto and fully auto method.

DISCUSSION:

Laboratory investigation are vital part of healthcare systems for diagnosis, prognosis and response to the treatment. Thus, maintaining the quality of any test results in a biochemical lab is crucial. Quality control helps to evaluate the analytical processes used in the clinical laboratories. The performance analysis of any test can be best described in terms of total analytical error which is summarized as performance standard. The test results are acceptable if they fall in allowable range of error limit and unacceptable if these show excessive error or are in out of the range. Therefore the standardization of instruments and the results obtained from multiple instruments should be reliable and in harmony. In this study, three biochemical parameters urea, creatinine and uric acid were compared for desirable limits of precision and accuracy for fully and semi-auto analyzers. Our study shows no significant difference (p-value>0.05) among the mean values of creatinine, urea and uric acid obtained from the fully and semi auto analyzers which is in accordance with the study of Chandana et al., 2003[2].

Kumari et al., 2020[9], Swetha et al., 2017[10] and Chandana et al., 2003[2] showed significant positive correlation for the parameters they have measured in semi and fully auto analyzer, which is in accordance with our study for all the three parameters urea (r value=0.9169), uric acid (r value=0.9178) and creatinine (r value=0.8896). Further in this study, Bland-Altman analysiss for urea, creatinine and uric acid shows that plot are evenly distributed along the line of no difference between the limits of agreements which is supported by Kumari et al., 2020[9] and Chandana et al., 2003[2].

According to European recommendation adopted by Baadenhuijsen et al., 2000[7] , Bias% were in acceptable limit for creatinine and uric acid but our study showed a significant difference for urea i.e. 7.0427, which is not acceptable. The difference in value of urea can be attributed to reaction time, incubation time factor generated and pipetting error while working on the semi-auto analyzer. This suggests that the readings obtained from semi-auto analyzer largely depend on the handling and accuracy of the analyst and are subject to infrequent differences as observed in our study. From the statistical analysis done in this study it can be suggested that both the analyzers can be efficient alternative of each other to provide proper diagnosis so that correct treatment can be given to the patients.

CONCLUSION:

This study conclude that the test results of biochemical parameters such as urea, uric acid and creatinine measured in semi-auto and fully-auto analyzers are highly relatable and comparable. The readings obtained from both the analyzers have significant positive correlation which suggest that both the machines are reliable alternative of each other and semi-auto analyzer can be used in case of breakdown of fully-auto analyzers, in primary health care centers and small scale laboratories.

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