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Research Article

Evaluation and comparison of automated analysers on hepatic enzymes

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ABSTRACT

Background: Clinical Biochemistry tests comprise over one third of all hospital laboratory investigation. The laboratory accreditation requirement has become an important aspect in selecting the analysers for analysing and evaluating the samples. Recently accrediting bodies are focusing on the importance of total quality management and assessment of trueness of laboratory measurements. The present study aimed to evaluate the hepatic enzymes using a single analytical methodology in 2 different automated analysers (semi autoanalyser and fully automated analyser) to understand the reliability of instrumentation on analytical methodology that would fit the laboratory performance standard.
Methods: A total number of 50 serum samples from adult patients requested for liver function tests at Shri Sathya Sai medical college and research institute were analysed. The samples were evaluated for hepatic enzymes on (Cobasmira) Autoanalyser and (Biosystems) Semi Autoanalyser using the same analytical methodology and the values were compared between the 2 automated analysers. Data analysis was done by appropriate statistical methods.
Results: No large differences were obtained in the values between the 2 automated analysers. Mean ± SD of each of the hepatic enzyme analysed by automated analysers were very close to each other indicating a minimum bias. Pearson's correlation and scattered diagram showed significant positive correlation at 95% confidence interval between 2 automated analysers.
Conclusion: The findings of this study confirm that both the automated analysers were reliable for evaluation of hepatic enzymes.

Keywords: Clinical biochemistry, Analytical methodology, Hepatic enzymes, Autoanalyser and semi autoanalyser

INTRODUCTION

Clinical biochemistry is one of the most rapidly advancing areas of laboratory and clinical medicine. The marked increase in the number and availability of laboratory diagnostic procedures have helped in the solution of clinical problems. Hepatocyte injury is commonly encountered in the practice of medicine and is often clinically silent until late in its course.[1] For this reason, laboratory tests are usually needed for recognition and characterization of the type of liver injury present.[1] Any injury to the liver that results in cytolysis and necrosis causes the liberation of various enzymes. The measurement of these hepatic enzymes in the serum is used to assess the extent of liver damage and to differentiate hepatocellular (functional) from obstructive (mechanical) disease. The most common enzymes assayed in hepatobiliary disease include alkaline phosphatase and the amino transferases.[2]

The aminotransferases are a group of enzymes that catalyze the interconversions of the amino acids and α-keto acids by transfer of amino groups. Aspartate aminotransferase (AST) also termed SGOT and Alanine aminotransferase (ALT) also termed SGPT are widely distributed in human tissues. AST is present in large

amounts in liver, renal, cardiac and skeletal muscle tissue. Increased levels are associated with liver diseases or damage, myocardial infarction, muscular dystrophy and cholecystitis.[3] Decreased levels are observed in patients undergoing renal dialysis and those with B_6 deficiency.[4-6] Monitoring the change in levels over a period of time is beneficial to the physician evaluating myocardial infarction or following chronic or resolving hepatitis. ALT is present in high concentration in the liver and to a lesser extent in kidney, heart, skeletal muscle, pancreas, spleen and lungs, making it more "liver specific". Increased levels are generally a result of primary liver diseases such as cirrhosis, carcinoma, viral or toxic hepatitis and obstructive jaundice. Decreased levels may be observed in renal dialysis patients and those with B_6 deficiency.

Alkaline phosphatase (ALP), involved in metabolite transport across cell membranes, is found in decreasing order of abundance, in placenta, ileal mucosa, kidney, bone, and liver. Physiologically elevated serum alkaline phosphatase occurs in pregnant women during the third trimester due to the isoenzymes of placental origin and in growing children during periods of bone growth. Increased levels of the enzyme occur in liver diseases (Hepatitis, Cirrhosis); most striking elevations occur in extrahepatic biliary obstruction, bone diseases when osteoblasts are more actively laying down osteoid (Rickets, osteomalacia and Paget's disease), Hodgkin's disease, secondary deposits in bone, particularly in the case of carcinoma of the prostate or congestive heart failure.[7,8] Transient elevation is seen during healing of bone fractures. Decreased levels occur in hypophosphatasia and malnourished patients.

A major role of the clinical laboratory is the measurement of substances in body fluids for the purpose of diagnosis, treatment or prevention of disease, and for greater understanding of the disease process. To fulfill these aims the data generated has to be reliable. Reliability of the selected method is determined by its accuracy, precision, specificity and sensitivity; with major emphasis of QC being laid on monitoring the precision and accuracy of the performance of analytical methods. Precision is the reproducibility of an analytical method and Accuracy defines how close the measured value is to the actual value.[9] It is the objective in very biochemical method to have good precision and accuracy. Specificity and Sensitivity refers to the ability of an analytical method to determine solely the analyte to be measured and to detect even small quantities of the measured analyte.[10] As new methods are developed they may offer improved detection limits which may help in the discrimination between normal results and those in patients with the suspected disease.

A number of factors, primarily preanalytical and analytical, affect the accuracy of test results. The key characteristics of any test are its bias and imprecision. Bias is primarily an analytical characteristic, in which reported results differ from the actual value. Imprecision, or lack of reproducibility, is due to both physiological and analytical factors. Two major types of errors may occur in a laboratory: (a) Random errors arise due to inadequate control on pre-analytical variables; patient identity, sample collection and labeling, handling and transport, measuring devices etc. (b) Systemic errors occur due to inadequate control on analytical variables; due to error in calibration, impure calibration material, unstable/ deteriorated calibrators, unstable reagent blanks etc.[10]

During the past few years, in clinical biochemistry there has been a considerable increase in clinical demand for laboratory investigations. When the volume of work increased, there arose a need for work simplification. Monostep methods are introduced to replace multistep cumbersome and inaccurate methods. Automation in clinical laboratory is a process by which analytical instruments perform many tests with the least involvement of an analyst. The function of autoanalyser is to replace with automated devices the steps of pipetting and increase the accuracy and precision of the methods. Instead of resorting to manual means automation leads to reduction in variability of results and error of analysis by doing away with jobs that are repetitive and monotonous for an individual and that can lead to boredom or casual attitude. However, the improved reproducibility attained by automation is not necessarily associated with improved accuracy of test results since accuracy is mainly influenced by the analytical methods used. The significant improvement in quality of laboratory tests in recent years is due the combination of well-designed automated instrumentation with good analytical methods and effective quality assurance programs.

The purpose of the study was to evaluate the hepatic enzymes using a single analytical methodology in 2 different automated analyzers (semi autoanalyser and fully automated analyser) to understand the influence of instrumentation on analytical methodology and familiarize with method selection and evaluation to fit the laboratory performance standard. A statistical analysis of the resulting data would indicate method bias, accuracy, precision and reliability of the respective methodology and techniques.

METHODS

Study population

The study group consists of 50 individuals who had requested for liver function test at the hospital central laboratory of Shri Sathya Sai Medical College and Research Institute. The study group was further divided into 2 groups based on the concentration of Hepatic Enzymes as shown in Table 1.

Table 1: Study group based on the concentration of hepatic enzymes.

Groups	Concentration of hepatic enzymes	Number of samples
Entire group	Normal and abnormal levels of hepatic enzymes (Group I + Group II)	N = 50
Group I	Normal levels of hepatic enzymes	N = 38
Group II	Abnormal levels of hepatic enzymes	N = 12

Sample collection and analysis

3ml of venous blood sample was collected in a plain tube. After centrifugation at 3000 rpm for 10 minutes, the serum samples were analyzed for hepatic enzymes using standard kits (ERBA) in Semi-Auto analyzer (Biosystems BTS 350) and Auto analyzer (Cobasmira) on the same day of collection.

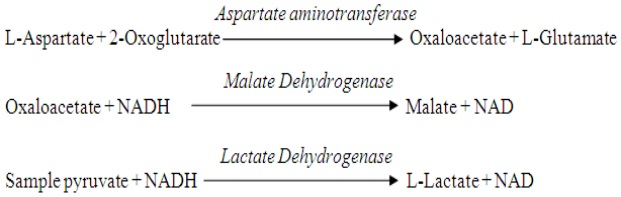
Biochemical methods

1) Estimation of SGOT

Method:

International Federation of Clinical Chemistry (IFCC method, kinetic)[11]

Principle:



Procedure:

Sample volume: 50µl
Reagent volume: 500µl
Temperature : 37°C
Wavelength : 340nm
Method : Kinetic
Linearity : Up to 450IU/L

Normal values:

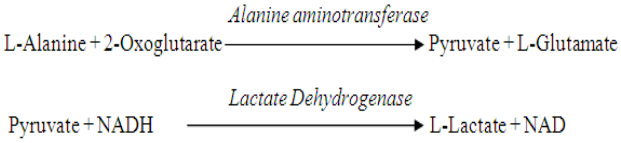
0-46U/L

2) Estimation of SGPT

Method

International Federation of Clinical Chemistry (IFCC method, kinetic)[11]

Principle



Procedure

Sample volume: 50µl
Reagent volume: 500µl
Temperature : 37°C
Wavelength : 340nm
Method : Kinetic
Linearity : Up to 450IU/L

Normal values:

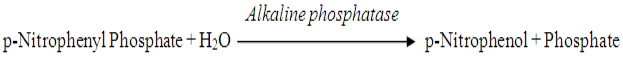
0-49U/L

3) Estimation of ALP

Method

Tris Carbonate Buffer (Kinetic)

Principle



Procedure

Sample volume: 10µl
Reagent volume: 500µl
Temperature : 37°C
Wavelength : 405nm
Method : Kinetic
Linearity : Up to 1000IU/L

Normal values:

40-125U/L

Statistical analysis

Data was expressed as Mean ± Standard Deviation. Correlation was calculated using Pearson's correlation Coefficient in the study group. P value <0.05 was considered statistically significant. All descriptive statistical analysis was performed using SPSS software, version 17.0.

RESULTS

The analysis of hepatic enzymes was studied under 2 groups: group I with normal levels of hepatic enzymes and group II with abnormal levels of hepatic enzymes. The values of hepatic enzymes obtained for each group

were compared between fully automated analyser (Cobasmira) and semi autoanalyser (Biosystems). Table 2 and Table 4 represent the comparison of hepatic enzymes between cobasmira & biosystems in group I. Table 3 and Table 5 represent the comparison of hepatic enzymes between cobasmira & biosystems in group II.

Table 2: Comparison of hepatic enzymes between cobasmira & biosystems in group I.

Hepatic enzymes	Group I				Correlation (r)	P value
	Cobasmira		Biosystems			
	Mean ± S.D	C.V.%	Mean ± S.D	C.V.%		
AST	24.45 ± 7.04	28.81	23.58 ± 7.00	29.71	0.982	0.00
ALT	22.74 ± 10.96	48.20	21.87 ± 10.60	48.47	0.993	0.00
ALP	87.21 ± 24.81	28.44	86.29 ± 24.76	28.70	0.999	0.00

Table 3: Comparison of hepatic enzymes between cobasmira & biosystems in group II.

Hepatic enzymes	Group II				Correlation (r)	P value
	Cobasmira		Biosystems			
	Mean ± S.D	C.V.%	Mean ± S.D	C.V.%		
AST	138.67 ± 62.17	44.83	137.58 ± 63.19	45.93	0.999	0.00
ALT	96.83 ± 37.87	39.11	95.67 ± 38.32	40.06	0.999	0.00
ALP	184 ± 25.33	13.76	184.33 ± 24.69	13.39	0.999	0.00

Table 4: Skewness and kurtosis of hepatic enzymes between cobasmira & biosystems in group I.

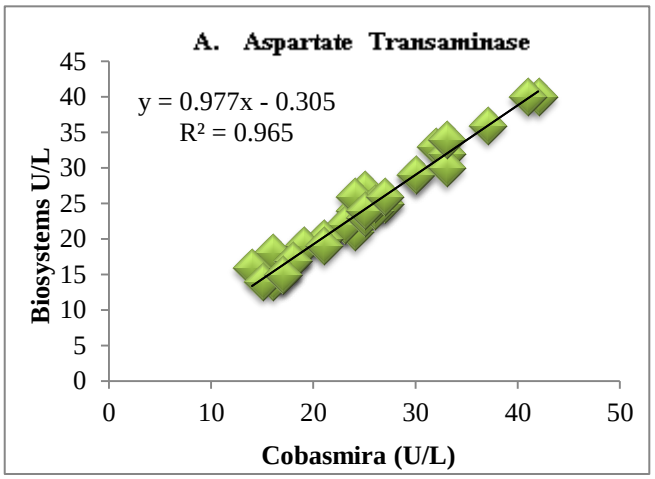
Hepatic enzymes	Group I			
	Cobasmira		Biosystems	
	Skewness	Kurtosis	Skewness	Kurtosis
AST	0.73	0.14	0.70	-0.06
ALT	1.26	1.44	1.30	1.62
ALP	0.86	0.41	0.89	0.44

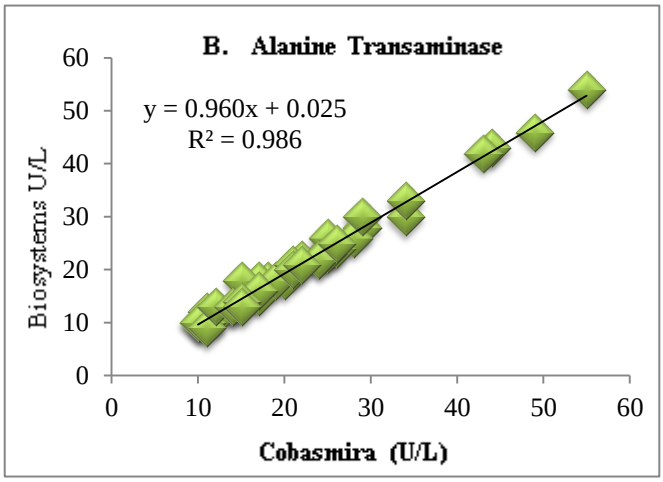
Table 5: Skewness and kurtosis of hepatic enzymes between cobasmira & biosystems in group II.

Hepatic enzymes	Group I			
	Cobasmira		Biosystems	
	Skewness	Kurtosis	Skewness	Kurtosis
AST	0.40	-0.57	0.43	-0.50
ALT	-0.04	-0.96	0.02	-1.06
ALP	-1.47	1.79	-1.46	1.97

Figure 1 and Figure 2 depicts the regression lines and the correlation of hepatic enzymes between fully automated analyzer and semi Autoanalyser in group I and II respectively.

In group I, comparisons were made on 38 serum samples analyzed for hepatic enzymes between cobasmira and biosystems analysers. Mean ± SD of each of the hepatic enzyme analyzed by automated analysers were very close to each other. As evident from table 2, only a minimum bias was obtained in group I by both the automated analytical instruments.





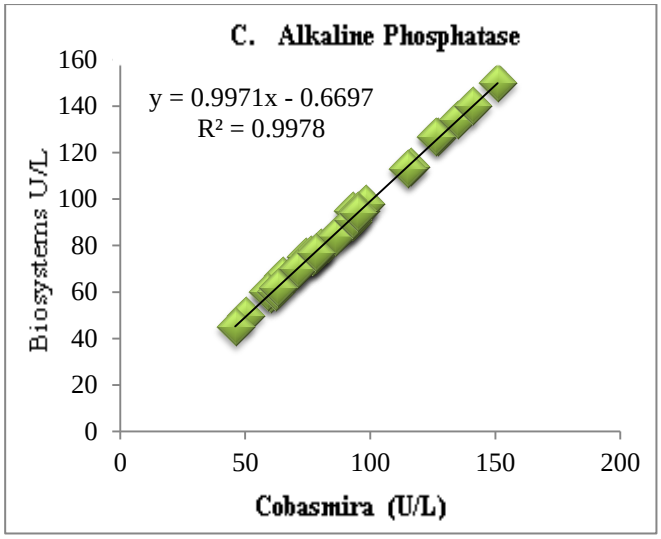


Figure 1: Scatter diagram depicting comparison of hepatic enzymes: cobasmira vs. biosystems in group I.

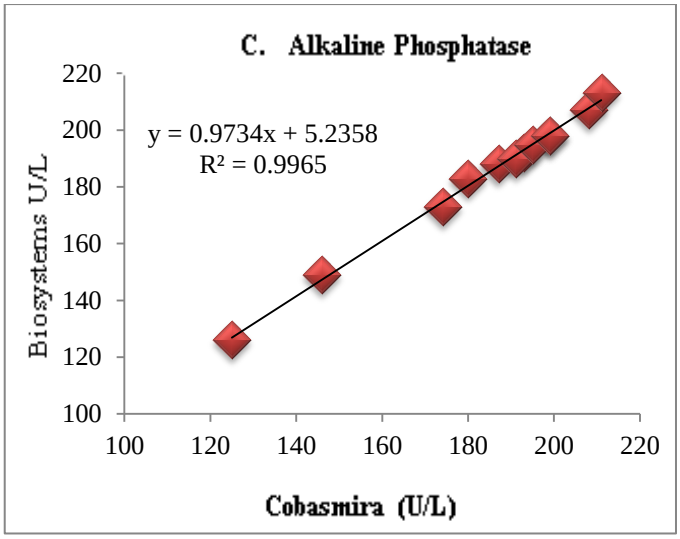
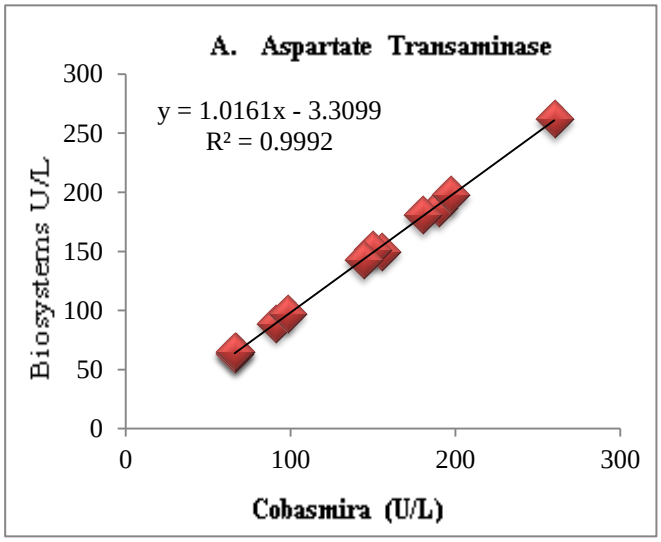


Figure 2: Scatter diagram depicting comparison of hepatic enzymes: cobasmira vs. biosystems in group II.



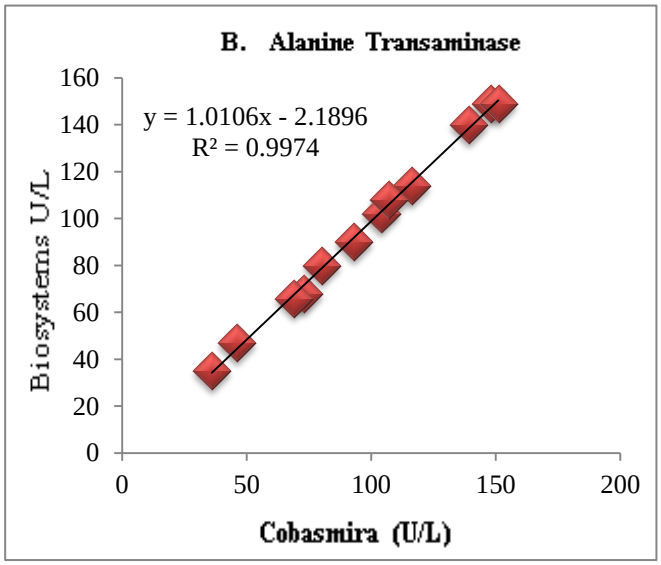


Table 2 and Figure 1 depicts the statistical analysis of hepatic enzymes in group I. AST gave a correlation coefficient of r = 0.982; P <0.05* at 95% confidence interval with a regression equation of y = 0.977x - 0.305 (where, y = activity of hepatic enzymes in biosystems, x = activity of hepatic enzymes in cobasmira). ALT gave a correlation coefficient of r = 0.993; P <0.05* at 95% confidence interval with a regression equation of y = 0.960x + 0.025 (where, y = activity of hepatic enzymes in biosystems, x = activity of hepatic enzymes in cobasmira). ALP gave a correlation coefficient of r = 0.999; P <0.05* at 95% confidence interval with a regression equation of y = 0.9971x - 0.6697 (where, y = activity of hepatic enzymes in biosystems, x = activity of hepatic enzymes in cobasmira). Hence, it can be summarized that the activity of hepatic enzymes in cobasmira and biosystems showed highly significant positive correlation.

In group II, comparisons were made on 12 serum samples analyzed for hepatic enzymes between cobasmira and biosystems analyzers. As evident from table 3, Mean ± SD of each of the hepatic enzyme analyzed by automated analyzers were very close to each other thereby, indicating only a minimum bias in group II also by both the automated analytical instruments.

Table 3 and Figure 2 depicts the statistical analysis of hepatic enzymes in group II. AST gave a correlation coefficient of r = 0.999; P <0.05* at 95% confidence interval with a regression equation of y = 1.0161x - 3.399 (where, y = activity of hepatic enzymes in biosystems, x = activity of hepatic enzymes in cobasmira). ALT gave a correlation coefficient of r = 0.999; P <0.05* at 95% confidence interval with a regression equation of y = 1.0106x - 2.1896 (where, y = activity of hepatic enzymes in biosystems, x = activity of hepatic enzymes in cobasmira). ALP gave a correlation coefficient of r =

0.999; P <0.05* at 95% confidence interval with a regression equation of y = 0.9734x + 5.2358 (where, y = activity of hepatic enzymes in biosystems, x = activity of hepatic enzymes in cobasmira). Hence, it can be summarized that the activity of hepatic enzymes in cobasmira and biosystems showed highly significant positive correlation.

As may be seen both from the slope and the intercept of the regression line and from the values in Table 2 and table 3, hepatic enzymes gave similar values in both the auto analyzers all through the experimental range of normal and abnormal concentrations in group I and group II.

As evident from Table 4 and Table 5; group I: the activities of hepatic enzymes analyzed in cobasmira indicate a leptokurtic curve and the activity of alanine transaminase and alkaline phosphatase analyzed in biosystems are also leptokurtic while that of aspartate transaminase is platykurtotic. The hepatic enzymes analyzed in both cobasmira and biosystems are right skewed. Group II: the activities of ast and alt analysed in cobasmira and biosystems were platykurtotic. On the other hand, ALP was leptokurtic and left skewed. Alanine transaminase was left skewed in cobasmira and right skewed in biosystems while AST was right skewed in both the analysers. All the statistical analysis performed was significant.

Both the automated analyzers have advantages and disadvantages. In the case of semi auto analyzers, the initial part of the procedure (pipetting of specimen and reagent, incubation) is carried out manually and the rest of the procedure is carried out by the analyzer in batches. The semiautoanalysers are cheap and compact, compared to other fully automated analyzers. In case of fully automated analyzer, the entire procedure is carried out by the analyzer and more than one reagent can be stored. Samples are placed in the machine and the computer is programmed to carry out any number of selected tests on each sample.

DISCUSSION

The International Union of Pure and Applied Chemistry (IUPAC) define automation as "the replacement of human manipulative effort and facilities in the performance of a given process by mechanical and instrumental devices that are regulated by feedback of information so that an apparatus is self-monitoring or self-adjusting". Presently no currently available clinical instrument fully meets this definition, however the term 'automation' is applicable to the individual steps in many analytical processes and modern instrumentation is improvising with more and more intelligence built into new generations of laboratory analyzers to come up to the IUPAC definition.

Automated instruments enable laboratories to process a much larger workload without a relative increase in manpower. When initially introduced, automation mimicked manual test procedures and was applied to those tests requested most often. Automation is a self-regulating process, where the specimen is accurately pipetted by a mechanical probe and mixed with a particular volume of the reagent and results are displayed in digital forms and also printed by a printer. There is an element of feedback which detects any tendency to malfunction. Automation may initially incur high costs for procurement of the equipment's but is economical in the long run due to the reduction in the manpower required to perform the tasks. The automated instruments not only save the labor and time but allow reliable quality control, reduce subjective errors and work economically by using smaller quantities of samples and reagents. Analytical methods, which are quicker and with fewer steps as well as modification of existing protocols are being developed as the manufactures have integrated computer hardware and software into analyzers to provide automatic process control and data processing capabilities.

To the best of our knowledge, this is the first study to report the effect of instrumentation on analytical methodology for evaluation of hepatic enzymes. The analysis of hepatic enzymes by both the automated analytical instruments indicates only a very minimal bias in the study group (Group I & group II) over various ranges of hepatic enzyme concentration. The values reported were similar in both the instruments, indicating good accuracy and precision as evidenced by the mean ± SD in the study group. Further, a highly significant positive correlation was obtained on comparison between the automated instruments in both the groups.

Despite the high costs involved in utilizing the automated instruments for analysis of hepatic enzymes; the minimum bias, accuracy, precision and good correlation obtained from this study strongly suggests both the automated analyzers to be highly reliable in the evaluation of hepatic enzymes in liver function test.

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