

# Comparative Study of Ethylenediaminetetraacetic Acid Plasma versus Serum for Assay of Biochemical Parameters

D Abhijith<sup>1</sup>, M D Nandini<sup>1</sup>, B G Vittal<sup>2</sup>

## ABSTRACT

**Objective:** The objective of the study was to assess the usefulness of ethylenediaminetetraacetic acid (EDTA) plasma as an alternative to serum for glucose, renal function test, and liver function test. **Need for the Study:** The use of plasma as compared to serum decreases the turnaround time, coagulation-induced interferences, gives high sample volume for analysis, and is economical. **Methodology:** An observational cross-sectional study was done on blood samples of 51 patients. A 4 ml of blood was drawn from each patient by standard venipuncture technique. A 2 ml was collected in a plain red top Vacutainer and 2 ml in an EDTA Vacutainer. Red top tubes, after 20 min of clotting, were centrifuged for 5 min at 5000 rpm to separate serum. EDTA tubes were centrifuged immediately at 5000 rpm to separate plasma. Serum and plasma were analyzed with ERBA-EM-360 autoanalyzer for glucose, urea, creatinine, total bilirubin, direct bilirubin, total protein, albumin, aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP). Results obtained were analyzed using paired “*t*-test.” **Results:** Values of glucose, urea, total bilirubin, direct bilirubin, total protein, albumin, and ALP in serum and plasma samples showed statistically significant difference with  $P < 0.05$ . Plasma glucose was more as compared to serum glucose. Creatinine, AST, and ALT values in serum and plasma samples showed statistically no significant difference with  $P > 0.05$ . **Conclusion:** EDTA plasma can be used as an alternative to serum for creatinine, AST, and ALT but its use for urea, total bilirubin, direct bilirubin, total protein, albumin, and for ALP may not be appropriate. **KEY WORDS:** Biochemical assay, ethylenediaminetetraacetic acid, liver function tests, plasma, renal function test, serum.

## Introduction

Serum is the specimen preferred for estimating the biochemical parameters by most of the laboratories. Serum is the liquid portion of the blood obtained after the blood has clotted completely. The Association of Physicians of India and American Diabetes Association (ADA) guidelines use plasma glucose for diagnosis of diabetes mellitus. Plasma is the cell-free supernatant layer obtained after centrifugation of the anticoagulated blood. Anticoagulants are the substances/compounds

that inhibit coagulation of blood. Majority of the laboratories are using serum as the specimen for almost all biochemical assays including glucose estimation. The results obtained using serum as specimen for glucose is being used to diagnose and monitor the treatment of diabetes mellitus by most of the physicians in our country.

The use of plasma as a specimen for biochemical assays offers many advantages as compared with the serum, the first one being adherence to the guidelines of ADA with respect to diagnosis of diabetes.<sup>[1]</sup> As the plasma samples can be centrifuged immediately after collection, it decreases the turnaround time. It gives 15–20% higher yield of volume than of serum that can be isolated from the same volume of blood. Its use also decreases the incidence of blockage of suction needles of analyzers by clots as commonly seen with serum samples.<sup>[2]</sup> Last but not the least, if plasma sample

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Doi: 10.46347/jmsh.2020.v06i02.009

<sup>1</sup>Tutor, Department of Biochemistry, Hassan Institute of Medical Sciences, Hassan, Karnataka, India, <sup>2</sup>Professor and Head, Department of Biochemistry, Hassan Institute of Medical Sciences, Hassan, Karnataka, India  
Address for correspondence:

Dr. B.G. Vittal, Department of Biochemistry, Hassan Institute of Medical Sciences, Hassan, Karnataka, India.  
Phone: +91-8904595910. E-mail: [vittal.bg@gmail.com](mailto:vittal.bg@gmail.com)

can replace serum for all biochemical assays, laboratories can avoid usage of plain clot activator tubes/Vacutainers, when multiple tests are ordered thereby reducing the economic burden on the laboratories and also making them ecofriendly as there is one less potentially hazardous tube per patient to dispose off.

There is variety of anticoagulants in the market with different mechanisms in inhibiting blood clotting. Hence, this study intends to know whether ethylenediaminetetraacetic acid (EDTA) plasma can be used as an alternative to serum for not only glucose but also for renal function test (RFT) which includes urea and creatinine and liver function test (LFT) comprising total bilirubin, direct bilirubin, total protein, albumin, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) as EDTA plasma Vacutainer/tubes are routinely used in the department of pathology for cell counts.

### Objective

The objective of the study was to assess the usefulness of EDTA plasma as an alternative to serum for glucose, RFT, and LFT.

### Methodology

An observational cross-sectional study was done on blood samples of 51 patients attending central laboratory of HIMS Teaching Hospital, Hassan. Patient selection was based on sampling of convenience, so patients were chosen irrespective of their clinical condition. Institutional Ethics Committee approval was obtained and consent of participants was obtained. A 4 ml of blood was drawn from each patient by standard venipuncture technique out of which 2 ml was collected in a plain red top Vacutainer and the other 2 ml in an EDTA purple top Vacutainer. Blood in red top tubes, after 20 min of clotting, were centrifuged for 5 min at 5000 rpm to separate serum. Blood in the EDTA tubes were centrifuged immediately at 5000 rpm to separate the plasma. Serum and plasma after separation were analyzed with ERBA EM 360 autoanalyzer using ERBA reagents for glucose (glucose oxidase-peroxidase method), urea (urease UV kinetic glutamate dehydrogenase method), creatinine (modified Jaffe's endpoint method), total bilirubin (Evelyn-Malloy's modified diazo method), direct bilirubin, total protein (biuret method), albumin (bromocresol green method), AST (IFCC-Kinetic

method), ALT (IFCC-Kinetic method), and ALP (p-Nitrophenyl phosphate AMP kinetic method). Routine quality control procedures were in place during the study. Results obtained were analyzed using paired "t-test" with SPSS statistical software. Paired "t-test" was used, as plasma is a substance obtained after adding an anticoagulant to serum. Results before and after addition of anticoagulant are compared using paired "t-test."

### Results

The study group comprised 51 patients of whom 22 were male and 29 were female of age groups, as shown in Table 1.

The values of glucose, urea, total bilirubin, direct bilirubin, total protein, albumin ALT, and ALP in serum and plasma samples showed statistically significant difference with  $P < 0.05$ . The values of plasma glucose were more as compared to serum glucose in almost all the samples.

The values of creatinine and AST in serum and plasma samples showed statistically no significant difference with  $P > 0.05$  [Table 2].

### Discussion

This study showed statistically significant difference in values between serum and EDTA plasma for urea, total bilirubin, direct bilirubin, total protein, albumin ALT, and ALP, thereby confronting the postulated hypothesis of use of EDTA plasma as a replacement for serum with respect to the above parameters.

Our study showed that there is no significant statistical difference in values between serum and EDTA plasma for creatinine and AST with  $P < 0.05$  warranting the use of EDTA plasma as an alternative for these two parameters.

**Table 1: Age and sex distribution of the study population**

Age group	Male	Female
21–30 years	4	1
31–40 years	7	3
41–50 years	6	7
51–60 years	7	5
61–70 years	3	2
71–80 years	2	4

**Table 2: Mean difference of analyzed parameters using serum and EDTA and their statistical significance**

Parameter	Serum sample*	EDTA sample*	P-value#
Glucose (mg/dL)	164±107	173±108	<0.0001
Urea (mg/dL)	20.9±6.9	20.6±6.7	0.0128
Creatinine (mg/dL)	0.9±0.27	0.9±0.25	0.4196
Total bilirubin (mg/dL)	0.84±0.48	0.76±0.45	<0.0001
Direct bilirubin (mg/dL)	0.36±0.19	0.32±0.18	0.0003
Total protein (g/dL)	7.2±0.5	7.1±0.5	0.0047
Albumin (g/dL)	4.1±0.4	4±0.4	<0.0001
ALT (IU/L)	21±39	23±37	0.0441
AST (IU/L)	26±27	25±26	0.0629
ALP (IU/L)	252±92	59±53	<0.0001

ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase. \*Mean±standard deviation.

#P<0.005 is considered statistically significant.

EDTA: Ethylenediaminetetraacetic acid

Kang *et al.* in their study published in 2016 in diabetes metabolism journal stated that when plasma glucose rather than serum glucose was used for diagnosis of diabetes, 20.9% of the subjects were newly diagnosed as diabetics.<sup>[3]</sup> Frank *et al.* in their study published in 2012 concluded that serum values of glucose were significantly lower than plasma values.<sup>[4]</sup> Holtkamp *et al.* in their study published in 1975 summarized that plasma glucose is significantly higher than the whole blood glucose independent of the method of pretreatment.<sup>[5]</sup>

Similar results were observed in our study. There is statistically significant difference between serum glucose and EDTA plasma glucose with  $P < 0.05$ , but when we compare the respective values in all patients, the value of plasma glucose is more as compared to serum glucose individually with mean plasma glucose of 173 mg/dL and the mean serum glucose level is 164 mg/dL suggesting that the plasma specimen is better than that of serum for glucose estimation which is in accordance with the previous studies and standard guidelines and hence can definitely replace serum.

Sharma *et al.* in their study observed that glucose, urea, creatinine, bilirubin, total protein, and serum glutamate-pyruvate transaminase can be assayed reliably from either serum or plasma. Their observations were not consistent with our study, however, their study analyzed the samples using dry chemistry platform.<sup>[6]</sup> Two studies have compared analyte concentration in heparinized plasma and serum, which differs from our study design and have given varied results.<sup>[7,8]</sup> Kamali and Mohri in a similar study demonstrated that concentration of total bilirubin, creatinine, AST, and ALT altered significantly between serum and EDTA plasma which is not consistent with our study results except for total bilirubin and ALT.<sup>[9]</sup>

With the above results, two parameters out of 10 studied can have either plasma or serum as a specimen, but the rest of the parameters are better estimated using serum itself, as serum is the preferred assay material for almost all assays unless specified. Plasma remains the preferred sample for glucose estimation. Larger study with a higher sample size can further validate the consistency and appropriateness of our study.

## Conclusion

EDTA plasma can be used as an alternative to serum for creatinine, AST, and ALT but its use for urea, total bilirubin, direct bilirubin, total protein, albumin, and for ALP may not be appropriate as per our study. Estimation of glucose in plasma will ensure that diabetes mellitus is not underdiagnosed, as plasma glucose levels are higher than serum glucose.

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**Financial Support:** None; **Conflicts of Interest:** None

**How to cite this article:** Abhijith D, Nandini MD, Vittal BG. Comparative Study of Ethylenediaminetetraacetic Acid Plasma versus Serum for Assay of Biochemical Parameters. *J Med Sci Health* 2020;6(2):46-49.

Date of submission: 2-07-2020

Date of review: 03-07-2020

Date of acceptance: 31-08-2020

Date of publication: 10-10-2020