Section: Biochemistry

Original Research Article

A STUDY TO COMPARE CHANGE IN EDTA AND SERUM PARAMETER OF DEGRADATIVE AND SYNTHETIC PANEL OF LIVER FUNCTION TEST

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Abstract

Background: Most laboratories prefer serum as the specimen when evaluating biochemical parameters. The liquid portion of blood that is collected after it has totally clots is called serum. The turnaround time is sped up since plasma samples can be centrifuged right away after collection. In comparison to serum that can be extracted from the same volume of blood, it yields a 15–20% higher volume yield. **Materials and Methods:** This comparative study will be conducted in Department of Biochemistry, Rama Medical College Hospital & Research Centre Kanpur. Sample taken from IPD& OPD patients received at Central research laboratory of Rama Medical College Hospital. **Result:** In the field of in vitro diagnostics, anticoagulants are used for preserving whole blood to perform a variety of haematological tests and to obtain plasma for coagulation and clinical chemistry analyses. Heparin is used for clinical chemistry, sodium citrate for coagulation tests, and ethylenediamine tetraacetic acid (EDTA) for hemocytometry. Although all age groups are presented with a high prevalence of Serum and EDTA, our study indicates that the value of Serum ALT and EDTA ALT is same and p-value is not less than 0.05 which is not considered as significant, Serum AST is lower than EDTA AST and p-value is less than 0.05 which is considered as significant and Serum ALP is more elevated than EDTA ALP and p-value is less than 0.05 which is considered as significant. Shows that In Total Protein Serum value is low whereas EDTA value in high, in Albumin both Serum & EDTA value are same, in Bilirubin Total Serum value is high & EDTA value is low and Bilirubin Indirect Serum value is low & EDTA value is high. The p-value of Total Protein, Albumin, Bilirubin Total, Bilirubin Indirect is not less than 0.05 which is not considered statically significant. **Conclusion:** Biochemical screening for Serum and EDTA is paramount importance in all liver patients, as well as in all patients with unexpected worsening of their liver profile or vice-versa because our data statistically suggest that the effect of Serum and EDTA is associated with liver disorders. From this study, it can be concluded that Serum is most common Sample in any aged subjects. So, clinicians should remain highly suspicious in middle aged subjects with liver profile for increase in atherogenic parameters which may enhance the risk for liver chriosis leading to hepatic artery disease. Therefore, treatment and follow-up of liver patients should include the monitoring of liver profile parameters, Serum in order to decrease the possible effect of changing in the level of these parameters on the risk of liver diseases. Liver function test can be used in daily routine laboratory assessment of most metabolic diseases especially in obese and liver patients. Thus, targeting liver in the treatment of metabolic diseases would be very appropriate.

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. 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 analysers by clots as commonly seen with serum samples.[1] Human plasma and serum are commonly used matrices in biological and clinical studies.[2] Serum is obtained from blood that has coagulated. Fibrin clots formed during coagulation, along with blood cells and related coagulation factors, are separated from serum by centrifugation.[3] Serum from coagulated blood is the preferred specimen for clinical chemistry analysis. But plasma obtained with an appropriate anticoagulant may be an equally valid specimen and in certain condition preferable to serum.[4] To obtain plasma, an anticoagulant like EDTA of heparin is added before the removal of blood cells. Several studies have examined the proteomic differences between plasma and serum.[5] 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 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.[6]

Ethylenediaminetetraacetic acid (EDTA) is an aminopolycarboxylic acid with the formula [CH2N(CH2CO2H)2]2. This white, water-soluble solid is widely used to bind to iron andcalcium ions. It binds these ions as a hexadentate ("six-toothed") chelating agent. EDTA is produced as several salts, notably disodium EDTA, sodium calcium edetate, and tetrasodium EDTA.[7] EDTA is used extensively in the analysis of blood. It is an anticoagulant for blood samples for CBC/FBCs, where the EDTA chelates the calcium present in the blood specimen, arresting the coagulation process and preserving blood cell morphology. Tubes containing EDTA are marked with lavender or pink tops EDTA.[8] In the field of in vitro diagnostics, anticoagulants are used for preserving whole blood to perform a variety of haematological tests and to obtain plasma for coagulation and clinical chemistry analyses. Heparin is used for clinical chemistry, sodium citrate for coagulation tests, and ethylenediamine tetraacetic acid (EDTA) for hemocytometry. The main property of EDTA, a polyprotic acid containing four carboxylic acid groups and two amine groups with lone pair electrons, is the ability to chelate or complex metal ions in 1:1 metal-EDTA complexes. Owing to its strong complexation with metal ions that are cofactors for enzymes, EDTA is widely used as a sequestering agent to prevent some enzyme reactions from occurring. When blood is collected with no additives within an appropriate container (blood tube), it clots fairly quickly. As calcium ions are necessary for this process, the specific association between the carboxylic groups of EDTA and calcium is a reliable solution to prevent clotting, stabilizing whole blood in a fluid form, as required for some laboratory analyses. EDTA was chosen for haematological tests when aniline derived dyes were proposed for preparing blood smears from peripheral venous blood. EDTA allows optimal dying with MayGrunwald Giemsa stain. Heparin, conversely, triggersplatelet (PLT) activation, is more expensive and affects the staining properties, producing a reddish coloration. Citrate is used as an anticoagulant primarily for coagulation studies. An investigation on the Sysmex CD4000 system counting (CBC), using corrections for different dilutions. Blood smears stained using the Wright method were similar to those prepared using EDTA anticoagulated blood.[9] Anticoagulants are commonly added to collection tubes to prevent clot formation in vitro and to maintain blood in the fluid state for haematological testing. Historically, ethylene diamine tetraacetic acid (EDTA) has been recommended as the anticoagulant of choice for haematological testing, since it allows the best preservation of cellular components and morphology of the blood cells.[10] Accordingly, blood collection tubes for haematological testing must be filled to the correct volume to ensure a proper blood-to- additive ratio, and adequately mixed to prevent clotting. Although the Clinical and Laboratory Standards Institute (CLSI) recommends that primary tubes that contains an additive should be gently mixed 5 to 10 times to allow a complete interaction between blood and anticoagulant,3 there is no evidence that alternative mixing procedures for primary samples would influence the results of haematological testing. This lack of reliable information prompted us to determine the influence of primary tube mixing on haematological testing and, in particular, the minimal amount of inversions required for the primary specimens immediately after collection to enable clinically-reliable results in haematological testing.[11] Serum is often considered the gold standard as it is obtained from blood that has been coagulated and requires no additives, whereas plasma is obtained by mixing blood with an anticoagulant to inhibit the blood from clotting, followed by collecting the plasma supernatant. While the choice of serum or plasma may depend on the specific research purpose, it may also depend on sample availability such as in clinical and epidemiological studies which routinely biobank biological samples for future analysis.[13] Liver Function Tests (LFTs) are one of the most

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commonly requested screening blood tests. Whether for the investigation of suspected liver disease, monitoring of disease activity, or simply as 'routine' blood analysis, these tests can provide a host of information on a range of disease processes. The title 'liver function tests' is, however, somewhat of a misnomer only the bilirubin and albumin given in this panel offer information regarding the functional capacity of the liver. At a basic level the evaluation of liver enzymes simply gives information as to whether a patient's primary disorder is hepatitic or cholestatic in origin. However, much more may be interpreted from these assays with knowledge of enzyme ratios and pattern recognition. This paper offers an insight to generalists of how to yield greater information from this simple test.[14] The liver enzyme profile should always be assessed in conjunction with a thorough history and clinical examination. Despite these invaluable tools, there are many occasions when doubt persists over an underlying diagnosis. For example, does an overweight diabetic who enjoys a few glasses of wine at the weekend have alcoholic or non-alcoholic fatty liver disease? In such circumstances the absolute liver enzyme levels and ratios may point the clinician in the right direction. Furthermore, the pattern of enzymes will assist, not only with differentiating between cholestasis and hepatitis, but will aid diagnosis when there is a mixed picture.[15] Liver has to perform different kinds of biochemical, synthetic and excretory functions, so no single biochemical test can detect the global functions of liver. All laboratories usually employ a battery of tests for initial detection and management of liver diseases and these tests are frequently termed as "Liver function tests", although they are of little value in assessing the liver function per se. Inspite of receiving a lot of criticism for this terminology, the phrase 'Liver function tests' is firmly entrenched in the medical lexicon. It might be argued that 'Liver injury tests' would be a more appropriate terminology. Moreover, the clinical history and physical examination play important role to interpret the functions. The role of specific disease markers, radiological imaging and liver biopsy cannot be underestimated.[16,17] It also referred to as a hepatic panel, are groups of blood tests that provide information about the state of a patient's liver. These tests include albumin, bilirubin (direct and indirect), and others. The liver transaminases aspartate transaminase (AST or SGOT) and alanine transaminase (ALT or SGPT) are useful biomarkers of liver injury in a patient with some degree of intact liver function.[18-20] Most liver diseases cause only mild symptoms initially, but these diseases must be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. This testing is performed on a patient's blood sample. Some tests are associated with functionality (e.g., albumin), some with cellular integrity (e.g., transaminase), and some with conditions linked to the biliary tract (gamma-glutamyl transferase and alkaline phosphatase). Because some of these tests do not measure function, it is more accurate to call these liver chemistries or liver tests rather than liver function tests. Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to detect the presence of liver disease, distinguish among different types of liver disorders, gauge the extent of known liver damage, and monitor the response to treatment. Some or all of these measurements are also carried out (usually about twice a year for routine cases) on those individuals taking certain medications, such as anticonvulsants, to ensure that the medications are not adversely impacting the person's livers.[21] The Purpose of my study is to assess the usefulness of EDTA plasma sample for the evaluation of in liver function test against serum sample for sample replacement as EDTA decreases the turnaround time (TAT) and coagulation time required for the serum sample to clot. This helps to give high sample volume for analysis and it is economical and reduces the amount of sample draw.

MATERIALS AND METHODS

Study area: This comparative study will be conducted in Department of Biochemistry, Rama Medical College Hospital & Research Centre Kanpur. Sample taken from IPD& OPD patients received at Central research laboratory of Rama Medical College Hospital.

Study period: The study duration was conducted from April 2022 to March 2023.

Sample Size: 100 sample will be taken in which 50 will be serum sample and 50 will be EDTA sample of same patients admitted at Rama Medical college hospital. They were divided into 2 groups:

I. 50 will be serum sample.

II. 50 will be EDTA sample of the same patients.

Inclusion Criteria

1. People & individuals who went to OPD for routine health check-up.
2. Sample of apparently healthly individuals.

Exclusion Criteria

1. Patients with endocrinological disorders.
2. Patients, renal insufficiency, pregnant women.
3. Also, acutely ill patients, patients on statins.
4. Participants with myeloproliferative disorders and in therapy with cytotoxic drugs, pregnant women, lactating mother, renal disorders and haemolytic disorders.
5. Patient with known case of liver disease.

Study Tool: A pretested questionnaire based on semi- constructed proforma was used as study tool to collect the data including basic profile of participants i.e age, sex, blood pressure and intake of any lipid lowering drugs.

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Consent: A verbal or written consent in their own native language was obtained from the participants before the sample collection.

Specimen collection: A 4 ml of blood was drawn from each patient by standard venipuncture technique under aseptic condition out of which 2 ml was collected in a plain red top Vacutainer and the other 2 ml in an EDTA purple top Vacutainer.

Specimen processing: Blood in red top tubes, after 20 min of clotting, were centrifuged for 15 min at 3500 rpm to separate serum. Blood in the EDTA tubes were centrifuged immediately at 5000 rpm to separate the plasma. Sample will be stored at -200C for long term stability of samples until assayed.

Investigation: In the present study the following analysis were conducted: Liver Profile Assay- Albumin, Total Protein, Bilirubin (Total & Direct), Alkaline Phosphatase (ALP), Alanine Transaminase (ALT/SGPT) and Aspartate Transaminase (AST/SGOT).

Statistical Analysis: All the parameters of two groups were analysed for mean and standard deviation. The results were expressed as Mean ± standard deviation. Data was analysed by statistical software SPSS Version 22.0. Comparison among two groups was done by using t- Test. Pearson's correlation coefficient was used to find the correlation between Serum and EDTA in liver function test.

RESULTS

The present research work included 100 Subjects (50 Serum and 50 EDTA healthy individuals). Gender and age distribution have been done to see the prevalence of Liver function test healthy among study subjects.

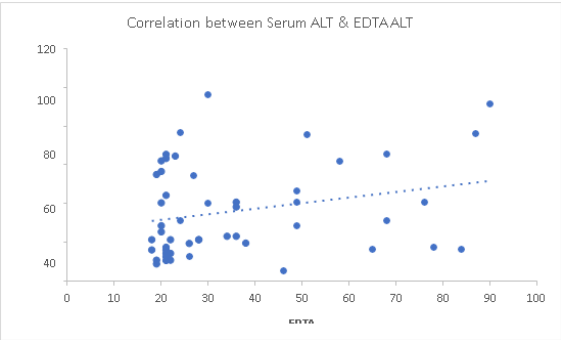

Figure 1: Scatter plot showing correlation of serum ALT with EDTA ALT.

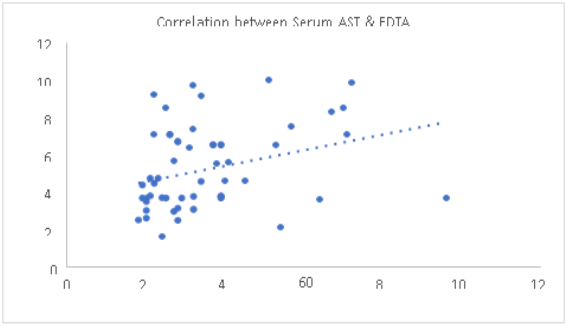

Figure 2: Scatter plot showing correlation of serum AST with EDTA AST.

[Figure 1] indicates a significant positive correlation between Serum ALT and EDTA ALT in cases (p-value in case of serum=0.26& in case of EDTA=1.00)

[Figure 2] indicates a significant positive correlation between Serum AST and EDTA AST in cases (p-value in case of serum=0.35 & in case of EDTA=1.00).

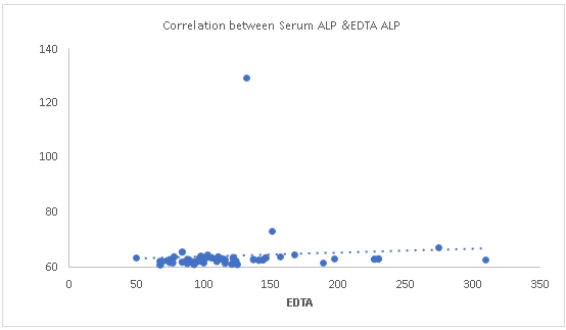

Figure 3: Scatter plot showing correlation of serum ALP with EDTA ALP

[Figure 3] indicates a significant positive correlation between Serum ALP and EDTA ALP in cases (p-value in case of serum=0.28 & in case of EDTA=1.00).

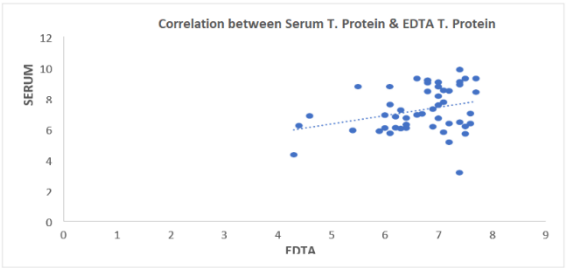

Figure 4: Scatter plot showing correlation of serum Total Protein with EDTA Total Protein.

[Figure 4] indicates a significant positive correlation between Serum T. Protein and EDTA T. Protein in cases (p-value in case of serum=0.25 & in case of EDTA=1.00)

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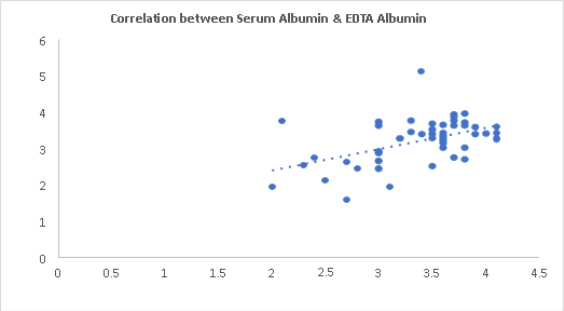

Figure 5: Scatter plot showing correlation of Serum Albumin with EDTA Albumin

[Figure 5] indicates a significant positive correlation between Serum Albumin and EDTA Albumin in cases (p-value in case of serum=1.00 & in case of EDTA=1.00).

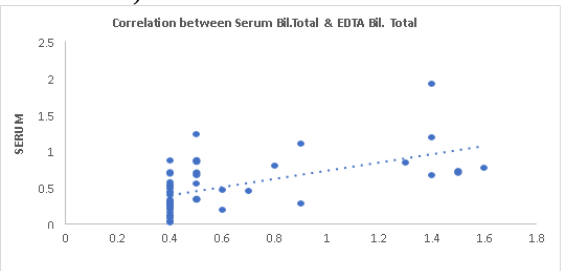

Figure 6: Scatter plot showing correlation of Serum T. Bilirubin with EDTA T. Bilirubin.

[Figure 6] indicates a significant positive correlation between Serum Bili. Total and EDTA Bili. Total in cases (p-value in case of serum=0.59 & in case of EDTA=1.00).

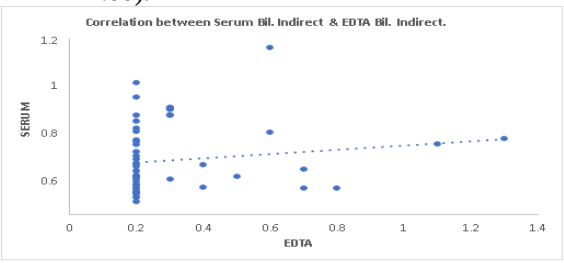

Figure 7: Scatter plot showing correlation of Serum Bili. Indirect with EDTA Bili. Indirect.

[Figure 7] indicates a significant positive correlation between Bili. Indirect and EDTA Bili. Indirect in cases (p-value in case of serum=0.20 & in case of EDTA=1.00)

Table 1: Analysis of variance between Serum and EDTA in Liver Function Test.

Parameter	Mean ±SD	t-value		p-value	
		SERUM	EDTA	SERUM	EDTA
ALT	36.11±23.65	-0.66	-0.66	0.94	0.94
AST	52.54±22.68	-4.14	-4.14	0	0
ALP	7.38±16.75	14.63	14.63	0	0
T. Protein	7.21±1.47	-2.29	-2.29	0.24	0.24
Albumin	3.38±0.52	0	0	1	1
Bili. Total	0.50±0.36	1.36	1.36	0.17	0.17
Bili. Indirect	0.37±0.23	-1.34	-1.34	0.18	0.18

DISCUSSION

The present study was conducted at Rama Medical College, Hospital & Research Centre, Kanpur, Uttar Pradesh, India with the objective to study to compare changes in EDTA and serum sample parameter of Liver Function Test and compare it with matched healthy individuals in the population. 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. 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 analysers by clots as commonly seen with serum samples. Ethylenediaminetetraacetic acid (EDTA) is an aminopolycarboxylic acid with the formula [CH2N(CH2CO2H)2].[2] This white, water-soluble solid is widely used to bind to iron and calcium ions. It binds these ions as a hexadentate ("six-toothed") chelating agent. EDTA is produced as several salts, notably disodium EDTA, sodium calcium edetate, and tetrasodium EDTA.[7] EDTA is used extensively in the analysis of blood. It is an anticoagulant for blood samples for CBC/FBCs, where the EDTA chelates the calcium present in the blood specimen, arresting the coagulation process and preserving blood cell morphology. Tubes containing EDTA are marked with lavender or pink tops EDTA.[8] In the field of in vitro diagnostics, anticoagulants are used for preserving whole blood to perform a variety of haematological tests and to obtain plasma for coagulation and clinical chemistry analyses. Heparin is used for clinical chemistry, sodium citrate for coagulation tests, and ethylenediamine tetraacetic acid (EDTA) for hemocytometry. Although all age groups are presented with a high prevalence of Serum and EDTA, our study indicates that the value of Serum ALT and EDTA ALT is same and p-value is not less than 0.05 which is not considered as significant, Serum AST is lower than EDTA AST and p-value is less than 0.05 which is considered as significant and Serum ALP is more elevated than EDTA ALP and p-value is less than 0.05 which is considered as significant. Shows that In Total Protein Serum value is low whereas EDTA value in

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high, in Albumin both Serum & EDTA value are same, in Bilirubin Total Serum value is high & EDTA value is low and Bilirubin Indirect Serum value is low & EDTA value is high. The p-value of Total Protein, Albumin, Bilirubin Total, Bilirubin Indirect is not less than 0.05 which is not considered statically significant. This is in accordance with the earlier studies that is Combined Serum was the most common parameter by most of the laboratories. Sharma et al. in their study observed that, Serum ALT, bilirubin, total protein, and can be assayed reliably from either serum or plasma. Their observations were not consistent with our study, however, their study analysed the samples using dry chemistry platform. Five studies have compared analyte concentration in heparinized plasma and serum, which differs from our study design and have given varied results. According to Kamali and Mohri in a similar study demonstrated that concentration of total bilirubin, AST, and ALT altered significantly between serum and EDTA plasma which is not consistent with our study results except for total bilirubin and ALT. But in our studies the p-value of AST, ALP is less than 0.05 which is statically more significant. In the present study, the mean ± SD levels of AST&ALP Liver function Test were significantly higher and statistically significant. With the above results, two parameters out of 7 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 AST & ALP estimation. A number of liver function test are available to test the proper function of the liver, (serum proteins, serum albumin, bilirubin (direct and indirect), ALT, AST, GGT, ALP, PT and PTT). Imaging tests such as transient elastography, ultrasound and magnetic resonance imaging can be used to examine the liver tissue and bile ducts. Liver biopsy can be performed to examine liver tissue to distinguish between various conditions; tests such as elastography may reduce the need for biopsy in some situations. Liver Biomarkers as well as the related in vitro diagnostic antibodies used for diagnosis being provided. From our study, it is observed that Liver function test patients parameter having elevated ALT, ASP, ALP, Total Protein, Albumin, Total Bilirubin and Indirect Bilirubin shows positive correlation with Serum and EDTA. Our findings are in consistent with the figures mentioned in local as well as in the international literatures of authors, Sharma. et al., Kamli and Mori. et al. who reported positive correlation of ALT, AST, ALP, Total Protein, Albumin, Bilirubin with Serum and EDTA. Liver function tests are blood tests used to help diagnose and monitor liver disease or damage. The tests measure the levels of certain enzymes and proteins in your blood. Some of these tests measure how well the liver is performing its normal functions of producing protein and clearing bilirubin, a blood waste product. Other liver function tests measure enzymes that liver cells release in response to damage or disease. In this study, it was also observed that in liver profile parameters i.e increased ALT, AST, ALP, Total Protein, Albumin, Bilirubin. Similar findings was reported by A Abhijith et al. Our study is limited by the limited number of patients attending only Rama Medical College Hospital and Research Centre, Kanpur U.P and also due to limited period. Additionally, the list of potential confounders for above enzyme assay disturbances is long which need to be studied in details and requires large population to reflect the correlation correctly.

CONCLUSION

These findings suggest that, the biochemical screening for Serum and EDTA is paramount importance in all liver patients, as well as in all patients with unexpected worsening of their liver profile or vice-versa because our data statistically suggest that the effect of Serum and EDTA is associated with liver disorders. From this study, it can be concluded that Serum is most common Sample in any aged subjects. So, clinicians should remain highly suspicious in middle aged subjects with liver profile for increase in atherogenic parameters which may enhance the risk for liver chriosis leading to hepatic artery disease. Therefore, treatment and follow-up of liver patients should include the monitoring of liver profile parameters, Serum in order to decrease the possible effect of changing in the level of these parameters on the risk of liver diseases. Liver function test can be used in daily routine laboratory assessment of most metabolic diseases especially in obese and liver patients. Thus, targeting liver in the treatment of metabolic diseases would be very appropriate. Hence, ALT, AST level may be used as risk factors for liver chriosis. On the other hand, EDTA plasma cannot be used as an alternative to serum for ALT, AST, ALP, Total Protein, Albumin, total bilirubin and direct bilirubin may not be appropriate as per our study. Estimation of ALT, AST, ALP & Bilirubin in plasma will ensure that liver diseases are not underdiagnosed, as EDTA levels are higher than serum. Estimation of Total Protein & Albumin can be done in both EDTA & Serum sample.

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