Study of the Effect of Storage on the Level of Bilirubin and Some Serum Proteins

Hussein K. A. Hussein
Department of Chemistry, College of Science, University of Karbala

(NJC)

(Receivedon 19/2/2007) **(Accepted for publication on 6/9/2007)**

Abstract:

Serum total protein (S.T.P.), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, and serum total bilirubin are of biological and chemical importance in the diagnosis and follow up in different diseases. A variety of changes in the concentration of these substances with the time of storage were found with time. This research studied the possible changes in the concentration of these substances with time.

The results showed a decrease in all the biochemical substances that measured in the research and the amount of decrease depends on the time. The decrease depends on the chemical type of each parameter. Aminotransferases enzymes showed the most significant changes with storage are noticed in enzymes.

It was found a negative relationship between the storage time and each of the measured biochemical parameters in serum. The changes in serum total bilirubin explained by the possible effect of light on the chemical transformation of bilirubin into lumirubin. Serum proteins, especially enzymes, decrease due to the possible denaturation caused by freezing and thawing processes. It is recommended that the analysis should be done immediately after blood aspiration. If the sample delayed then the results should corrected according to the ratios that obtained in this work.

Keywords: Aspartate aminotransferase, AST, alanine aminotransferase, ALT, albumin, S.T.P., and bilirubin.

الخلاصة

يعتبر الألبومين والبروتين الكلي والبيليروبين الكلي وانزيمي الانين امينوترانسفريز و اسبارتيت امينوترانسفريز في المصل من المواد ذات الأهمية من الناحية الكيمياوية و الحياتية في تشخيص ومتابعة مختلف الامراض. وقد وجدت أنماط متنوعة من التغيير في تركيز هذه المواد بمرور الفترات الزمنية المختلفة (2,1، 3، 4 ، 5، 7,6) يوما. تهدف هذه الدراسة إلى دراسة العلاقة بين أنماط التغيرات المحتملة في مستويات المواد الموضحة أعلاه في المصل مع الزمن.

اظهرت النتائج انخفاض في نتائج كل المواد المقاسة مع الزمن اعتمادا على نـوع المـادة وفتـرة الخزن. كانت الانزيمات المقاسة هي اكثر المواد تاثرا بالخزن. استنادا للتحاليل المختبرية هنالك تأثير للخزن على نتائج في مصل الدم ويكون هناك انخفاض في نتائجها مع الزمن. فسر انخفاض البيليروبين الكلي بتأثير

الضوء عليه بتحويله كيميائيا الى لوميروبين. أما الانخفاض في نتائج تركيز البـروتين الكلـي وخـصوصا إنزيمي الانين امينوترانسفريز و اسبارتيت امينوترانسفريز في المصل فيمكن أن تعزى الى مسخ البروتينـات نتيجة لعملية الانجماد والاذابة للمصل المحفوظ بالتجميد.

ويوصى بإجراء التحليل المطلوب مباشرة بعد سحب العينة. وإذا تأخرت العينـة فيجـب أن تؤخـذ النسب المستخرجة من هذا البحث بنظر الاعتبار لتعديل النتائج إلى قيمها الحقيقية.

مفاتيح الكلمات، البيليروبين، أنزيم، الانين امينوترانسفريز ،اسبارتيت امينوترانسفريز.

Introduction

To obtain exact laboratory data that show or reflect the path physiological conditions of patients, preanalytical, analytical and postanalytical processes should be checked. However, it is assumed that preanalytical issue is one of the weak points in current laboratory medicine [(1)]. In the daily routine work in clinical laboratories, the storage of some biological samples (e.g. serum, urine, and other body fluids) may be required for different reasons. In many hospitals, the blood may be stored for different intervals due to different reasons such as for forensic examination [(2)]. Hence, there is a gap between the blood aspiration and the measurements. This delay affects the results and subsequent judgment obtained from these results for diagnosis, treatment, and follow up. In many cases, some samples have been received to the laboratory later in the night, some reagents may be exhausted, and some samples may be transferred into other laboratories for certain analysis that is not present in the laboratory. In addition to the fact, some patients are unable to come to the laboratory due to their severity of illness. Hence, the blood aspiration may carry out at their houses and transferred into the laboratory. Sometimes immediate sample separation or rapid transport of chilled blood samples to a central laboratory may be impractical or prohibitively expensive.

In Iraq, there is another chronic problem that may be not found in other countries; the electricity supplied may be shutdown at any time during the analysis for prolonged time. For these reasons, the storage of biological samples especially serum is required. It is well reported that many results of analyses concerning abnormal laboratory data produced by inadequate handling of samples and sample collection. Blood storage time may, along with other risk factors, play a significant role in blood transfusion-associated development of postoperative infectious complications [(3)].

Some studies were carried out on the effect of storage on animal blood components [(4)] because the time lost between the aspiration and measurement in veterinary clinics. In the present work, the human sera are used to study the effect of storage.

Frozen serum samples may thaw without mixing and various layers having different concentrations formed and contained. In one research, sixty items tested. Comparing the concentration before freezing, the upper layer contained half and the bottom two times more. Handling with the stored serum by preheating sera at 56°C for 30 minutes reduced markedly the levels of alanine aminotransferase, alkaline phosphatase, creatine kinase, and choline esterase. In such sera, temperature management is important. Scientific data on the storage and collection of samples should be informed not only to laboratory staffs but

to nurses and doctors in order to provide "exact" data [1]

In order to obtain accurate results, the analyst should know the possible biochemical changes (qualitatively and quantitatively) that occurred if he had to store the serum for different intervals. This work tries to compute the percentage of changes in the concentration of some biochemical substances in serum and introduce a correction factors to correct the obtained values.

There are different studies concerned with effect of storage on different biochemicals in blood or serum such as hormones [5], different steroids [6] and electrolyte levels that showed different changes during blood storage [7].

Storage of serum also found to affect the techniques and modify the results of enzyme-linked immunosorbent assay (ELISA) protocols [8], polymerase chain reaction [9], DNA extraction [10] and molecular detection of circulating prostate cells in cancer .

There also recorded hemorheological and morphological changes in blood during storage under standardized conditions for different intervals analyzed by sequential determination of the variability of aggregation, deformability, and shape of erythrocytes [11] and reduce the formation of proinflammatory membrane breakdown products [12]. Blood storage also has different effects on the erythrocyte sedimentation rate [13].

The combination of serum total bilirubin (STB), in addition to other parameters may identify infants with inceased bilirubin production (e.g., hemolysis) or decreased elimination (conjugation defects) as well as infants who require early follow-up after discharge for jaundice or other clinical problems such as late anemia [14].

Measurement of total serum protein (S.T.P.) content provides general information reflecting disease state in many organ systems. S.T.P. and albumin found to be decreased in different cancers [15] and in malnutrition [16] .

Many studies focused on the effect of storage time and conditions on the measurement of different blood parameters and different results were obtained [17]. Hence, serum parameters that selected for the present work were chosen because they are routinely measured. Most of these parameters are of diagnostic or prognostic value. Alanine aminotransferase (ALT) (EC 2.6.1.2) and bilirubin are important parameters in different types of hepatitis [18]. The parameters have been chosen in this research are important in the evaluating of hepatotoxicity by measuring the activity of serum enzymes, aspartate aminotransferase (AST) (EC 2.6.1.1), and ALT, as well as serum total bilirubin level [19]. Furthermore, measurement of ALT, AST and bilirubin are important in obstructive jaundice and its nutritional complications [20].

The goal of the present work is to give a factor can be used to correct the measured values (if present) depending on the time of delay between aspiration of blood and laboratory work.

Materials and Methods

Ten milliliters of venous blood samples were collected from ten healthy college students aging 20 years old who do not on any medications. Sera were separated and each serum samples distributed into aliquots of 0.5 milliliters in seven tubes and stored at refrigerator temperature. Serum AST, ALT, albumin, S.T.P., as well as serum total bilirubin level were measured at different intervals (1, 2, 3, 4, 5, 6, and 7 days).

Serum total bilirubin measured using Biomareux® kit. S.T.P. measured by biuret method. Albumin measured by bromcresol green method according to the manufacturer instruction of Randox® kit. Serum AST and ALT were measured colorimetrically using Randox® kit.

Results

The following results of the experiments are obtained and expressed as Figures (1-5) and Tables (1 and 2). Figure (1) indicates a decrease in serum total bilirubin at different intervals and a maximum decrease occurred after the

seventh days. Figure (2) showed a decrease in S.T.P. with time. Figure (3) showed a decrease in serum albumin at different intervals. The results showed a maximum decrease after seventh days. Serum ALT and AST showed the highest decrease at different intervals as noticed in Figure (4 and 5) respectively.

Table (1) shows the factors that the results of analysis should be multiplied to obtain accurate results after elapsing of different time intervals. The values of factors were obtained by division of the concentration of serum parameter that measured immediately (the correct value) on its concentration after elapsing different intervals. The percentage of deficiencies in the measured serum analytes after elapsing of different intervals are introduced in Table (2). The percentage of deficiencies were calculated from the simple formula=(1-Correct concentration/Concentration after storge)X100%). The most significant changes with storage are noticed in aminotransferases enzymes

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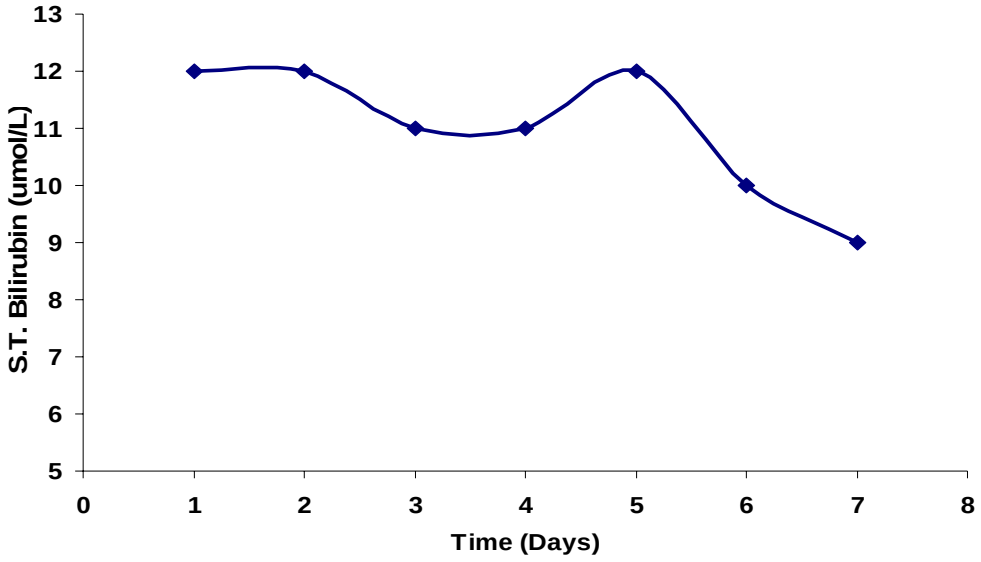

Figure (1): Change in serum total bilirubin level with time.

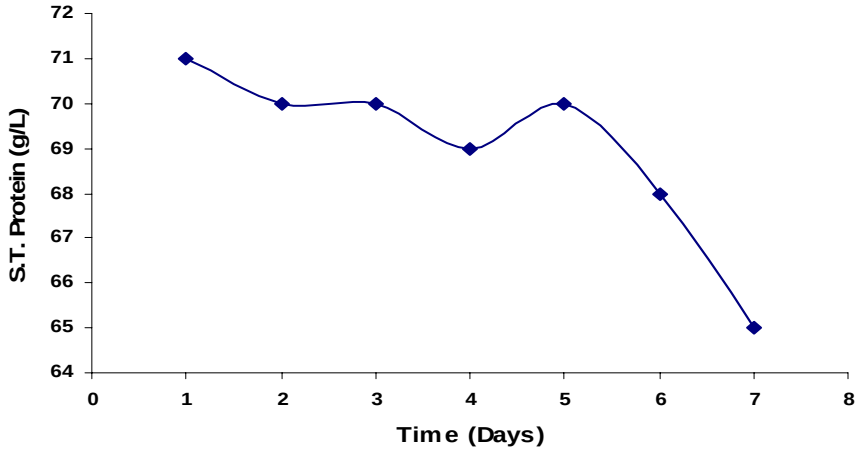

Figure (2): Change in serum total protein level with time

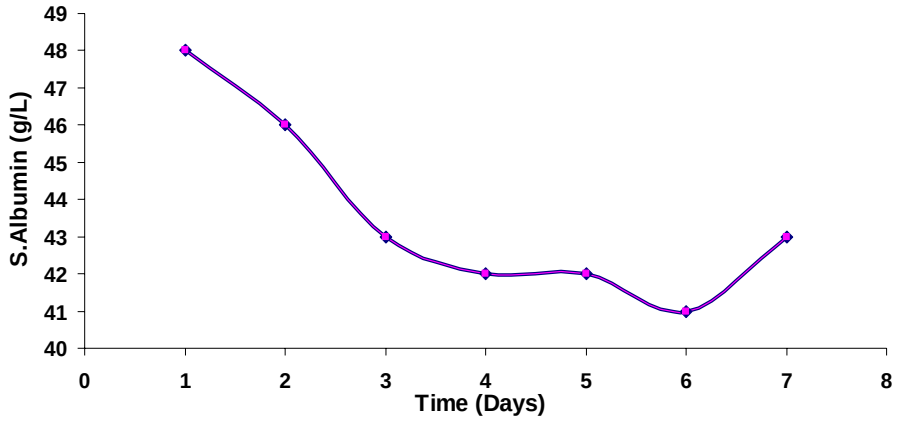

Figure (3): Change in serum albumin level with time.

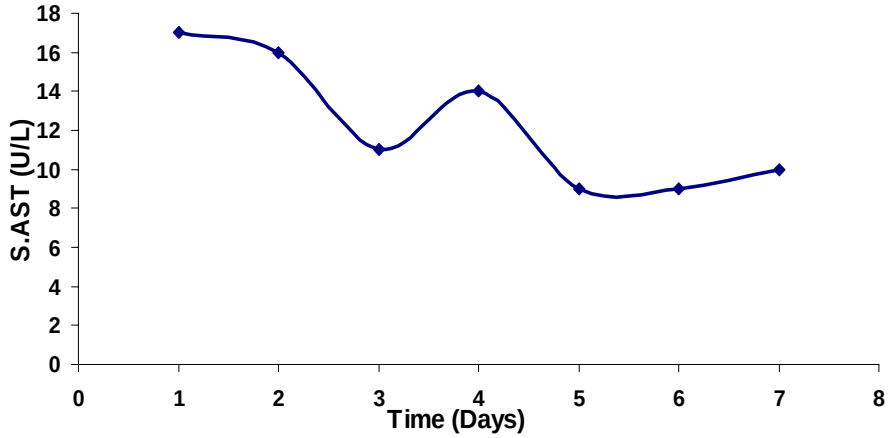

Figure (4): Change in serum aspartate aminotrasferase (AST) level with time.

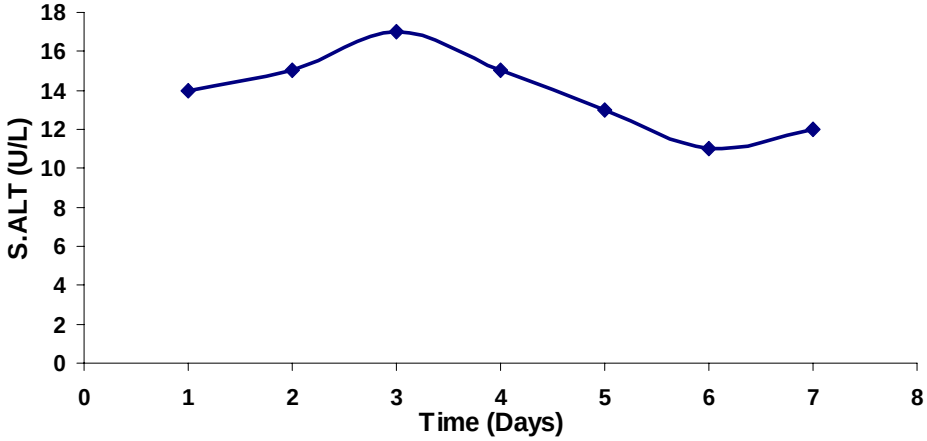

Figure (5): Change in serum alanine aminotrasferase (ALT) level with time.

Table (1): The factors that the results of analysis should be multiplied to obtain accurate results after elapsing of different time intervals.

Days	Bilirubin	T.S.P.	Albumin	ALT	AST
1	1.00	1.00	1.00	1.00	1.00
2	1.13	1.01	1.00	0.89	1.00
3	1.13	1.01	0.98	1.00	1.60
4	1.31	1.03	1.04	1.09	2.00
5	1.42	1.01	1.02	0.96	3.20
6	1.70	1.04	1.06	1.67	2.67
7	1.89	1.09	1.09	2.08	3.20

Table (2): Percentage of deficiencies in some serum analytes after elapsing of different intervals.

Days	Bilirubin	T.S.P.	Albumin	ALT	AST
1	0.00	0.00	0.00	0.00	0.00
2	11.77	1.41	0.00	-12.00	0.00
3	11.77	1.41	-1.96	0.00	37.50
4	23.53	2.82	3.92	8.00	50.00
5	29.41	1.41	1.96	-4.00	68.75
6	41.18	4.23	5.88	40.00	62.5
7	47.06	8.45	7.84	52.00	68.75

Discussion

The results of the present work are decreased in all the measured serum components. Some of the present results are agreed while other results are not agreed with the results of other researchers as discussed in the following paragraphs.

There is a known effect of light on the samples for the estimation of total bilirubin values *in vitro* [21]. Leung *et al* (1992) [22] concluded that blood samples for total bilirubin values should not be placed under the light of phototherapy even 2 hours. If blood samples are exposed to room light inevitably, it is safe to be checked within four hours. If immediate measurements are unavailable, the samples can be placed in a dark environment allowing the values to remain unchanged for 48 hours. Furthermore, it is re-emphasize the importance of shielding serum from light to avoid generating bilirubin photoproducts that interfere with the accurate determination of serum bilirubin subfractions [23].

Phototherapy is the most common form of therapy of jaundice [24] to decrease bilirubin into lumirubin, which is easily excretable. Three photochemical reactions are of importance in this treatment: photooxydation of bilirubin followed by fragmentation of the molecule and formation of water-soluble products (lumirubin and its intermediates) [25].

In one research, S.T.P. samples were unchanged for 7 days [26]. This fact confirms that the freezing and thawing processes and subsequent protein denaturation are the major cause for the decrease in S.T.P. noticed in this work.

Storage also affects polypeptides such as complements [27] and different types of antibodies titers values were also affected by the length of storage time [28] subsequently may affect collectively the S.T.P.. Whereas there is a report of apparent increase in albumin concentration, for samples stored for years, possibly could be attributed to an

unfolding of the protein, allowing more bromocresol green to be bound [29].

In one research, whole blood stored at room temperature for up to 7 days, concentrations of albumin, cholesterol, high-density lipoprotein (HDL), S.T.P., and triglycerides changed by less than 4%. Whilst ALT, creatine kinase (CK), creatinine, and gamma-glutamyl transferase (γGT) concentrations changed substantially at room temperature, there was less than 4% change during chilled storage up to 7 days. By contrast, AST concentration increased markedly under both conditions [30].

The results in Table (1) showed the factors that the results must be multiply with in order to obtain a correct values. Hence if some analytes delayed for few days, the values obtained should be multiply with these factors to obtain correct values. The results that sowed an increase in the concentration after elapsing of time should be ignored because they are included within the personal errors of measurements. Table (2) revealed the percentages of deficiencies in some serum analytes after elapsing of different intervals. This table is useful in the estimating of the most parameter that affected with the storage. From this table it can be concluded that aminotransferases are the most affected parameters than others. This fact can be explained depending on the fact that these parameters are enzymes and present in serum in very low concentrations as compared with other measure parameters. Denaturation occurred by freezing and thawing because of the sensitivity of protein structures for the freezing and thawing process.

The values of S.T.P., AST, γGT, alkaline phosphatase, bilirubin, cholesterol, urea, albumin, and electrolytes varied in all the sample substrates investigated at 20-22 ° C and 4 ° C less than +/- 10% during the observation period of up to four days [5]. The storage characteristics of nine enzymes (ALP, ALT, amylase, AST, CK, γGT, LDH, lipase), and 15 metabolites and minerals including (albumin, bilirubin, and S.T.P.) were studied. Parallel samples of serum and heparinized plasma were stored for 90 and 240 days at two different storage temperatures, -20°C and -70°C. Sixteen of the 24 analytes including (ALP, ALT, AST, bile acids) showed statistically significant ($p < 0.05$) changes during the storage period related to storage time, storage temperature, and sample type [31].

The increase in serum level of some blood components at third, fifth, or seventh day of storage as noticed in the plotted figures can be explained as a result of different causes. The human error in analysis and the statistical calculation (because mean values were used in the plotting of the figures) can explain these abnormal points in the figures but they have no effect on the direction of the slope which give a decrease in serum component values as time elapsed. Many researchers consider the differences in many results were thought to be due to laboratory variability [32] i.e., there is a standard error in the values depending on the kits, laboratory staff, temperature, electrical current stability, ..etc. affects the results of the measured parameters.

Other workers compared the differences between results of serum aliquots assayed immediately for 12 constituents and frozen aliquots accumulated and assayed on a single day with the results of control serum variation from the same period. One aliquot of each weekly sample was stored frozen. Storage at -20 ºC for 15 weeks had a mild destructive effect on two enzymes in serum. In the other 10 constituents tested, comparison of variances indicated that long-term (weeks) variation in control serum assays is similar to the difference of variation between aliquots assayed immediately and those frozen and assayed at the same time [33].

Conclusions and Recommendations

The investigation for serum; S.T.P., AST, ALT, albumin, and serum total bilirubin gives false results when the serum stored for different intervals. It was found a negative relationship between the

storage time and each of the measured biochemical parameters. This may lead to misleading clinical diagnosis, treatment, and follow up of the patients. The best method, as a first step to reduce the changes in the measurements, is to separate serum/plasma from clot/cells as promptly as possible to achieve improved stability for most analytes under test.

It is recommended that the analysis should be done immediately after blood aspiration. If the sample delayed then the results should corrected according to the ratios that obtained in this work. Addition of a protein-stabilizing agent may be useful for storage of samples.

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