J.Basrah Researches (Sciences) Vol.32. Part.2. 77 - 81 (2006)

Effect of Storage on Some Blood and Serum Constituents

**Lamia M. Al Naama, Jamal Ahmed Abdul- Barry,
And Abdul – Qader Abdul – Wahab**

***Department of Biochemistry, College of Medicine,
University of Basrah, Basrah, Iraq.***

**ISSN –1817 –2695
Received 11/12/2005 , Accepted 25/6/2006**

Abstract:

Objective:

To examine the possibility that the storage of blood or serum can cause an effect on some blood parameters, the concentrations of total cholesterol (TC), urea, uric acid, Sodium (Na^+), potassium (K^+) and chloride (Cl^-) were determined in the blood or serum samples, stored at 4^oC, during 0, 2, 4, 6 hours as well as at 1, 2 and 6 days.

Key words: Storage, blood, serum, constituents.

Method:

A total of 56 subjects (35 males and 21 females), mean of age was 21 years (range:19-23 years) were selected for the study. They were non smokers, apparently, healthy persons, their weights within normal range, and they had no family history of diseases and no drug had been taken in last week. Blood samples were collected after an average fasting for 12 hours, and the concentrations of TC, urea, uric acid, Na^+, K^+ and Cl^- were determined immediately (i.e the zero time) and then later after 2,4,6 hours and 1,2 and 6 days. All samples were stored at 4^oC,

Results:

The passage of the time affects the concentration of many blood constituents once it has been shed from the body. In the serum sample and during 6 days of storage at 4^oC, the concentrations of TC and electrolytes (Na^+, K^+ and Cl^-) approximately remained constant. However, the concentrations of urea and uric acid were non – significantly lowered. In plasma, analysis was made immediately after blood collection and after 2,4,6 and 24 hours. A significant increase in the concentration of potassium ion and non– significant decreases in the concentration of urea and uric acid were observed during the storage of whole blood in a plane tube at 4 ºC for 24 hours.

Conclusion:

Based on these results, we can conclude that a contact between red blood cells and plasma stored at 4^oC for an over night or even a short period, may produce changes in many blood parameters.

Introduction:

Blood is a fluid substance that circulates in arteries and veins of the body. It is composed of plasma, which constitutes 55% of the volume of the blood and millions of cells, which constitute about 45% of its volume[1]. The total blood volume is about 5 liters in average[1]. Plasma is a complex substance, its principal component is water. It also contains plasma protein, inorganic substances such as sodium, potassium, calcium, chloride, carbonate and bicarbonate, sugars, hormones, enzymes, fats, amino acids and such waste products as

urea and creatinine[2]. On the other hand serum is the fluid obtained after the removal of one of plasma proteins components i.e fibrinogen.

Since blood is the major circulating fluid in the body, and it cannot be compensated by another substance, whether natural or synthetic[1]. The subject of blood storage has got an increasing importance, and it has got a lot of attention. As well, the analysis of the blood constituents has an important role in the diagnosis of diseases, and since the immediate analysis of the blood sample collected may not be always possible, the idea of the blood storage and its effect on different constituents of the blood become very important for right diagnosis[3].This study was done to assess the effect of the storage at 4^oC for a variable time on different blood constituents.

Method:

This study was conducted during the period of September 2001 until the end of February 2002. A total of 56 (35 males and 21 females), mean of age was 21±2 years (range: 19-23 years), were selected for the study. They were non-smokers, apparently healthy, their weights within normal range, and they had no family history of diseases and no drug had been taken in the last week. The participants were mainly selected from undergraduate students at the College of Medicine, University of Basrah.Ten ml of venous blood-samples after an average fasting of 12 hours were drawn through a sterile disposable syringe. Serum was immediately separated by low-speed-centrifugations, and the concentrations of TC, urea, uric acid, Na^+, K^+ and Cl^- were determined immediately (i.e the zero time) and then later after 1,2 and 6 days of storage at 4^oC, other samples of 10 ml were drawn by venipancture for the determination of all the above parameters after the contact between red blood cells and plasma for 2,4,6 and 24 hours. All samples were stored at 4^oC.The biochemical parameters were performed in the Department of Biochemistry, College of Medicine, University of Basrah. Serum cholesterol, urea and uric acid were determined by standard methods using kits from BioMerieux, France. Serum sodium and potassium concentrations are measured using Flame Emission Photometry Method[4]. Serum chloride levels the determined by Coulometric Titration Method using Chloride meter Coming EEL. Quality control sera from BioMerieux were included in each assay batch for the all above data. The results values were presented as mean±SD. Student "t" test was used for comparison of data. For all analysis, a value of 0.05 was considered significant.

Results:

Characteristics of all subjects who participated in this prospective study are summarized in Table 1. The effect of the storage on the concentrations of total serum cholesterol, urea, uric acid, sodium, potassium and chloride in a serum sample is presented in Table 2. During 6 days of storage at 4^oC,, the concentrations of total cholesterol and electrolytes are approximately remained constant, however, the concentrations of urea and uric acid were non-significantly lowered when compared to their values on the zero time and on the first day ($P>0.05$).

Table 3 shows the effect of the storage at 4^oC, on the concentrations of TC, urea, uric acid and electrolytes in the plasma in contact with RBC for a period of 2,4,6 and 24 hours.

A significant increase in the concentration of potassium ($P<0.05$) and non-significant decreases ($P>0.05$) in the concentrations of urea and uric acid were observed during the storage of whole blood in a plane tube at $4\ ^oC$. This increase was approximately more with the increase in the storage of time (i.e 24 hours). However, the concentrations of TC, Na^+ and Cl^- remain approximately constant.

Discussion:

By examining the data in Table 2 we can observe that in serum sample, the electrolytes and cholesterol were not affected by time, since their levels remained approximately constant. The other two components showed non-significant decreases in their levels. This is mostly due to inappropriate storage conditions. Regarding urea, it might be unstable under storage conditions, due to its high content of nitrogen, so a small amount of it may be converted back into ammonia[5]. The uric acid also showed a decrease in its

concentration, although the reason of this decrease may also be due to the conditions of the storage, but it may affect it differently, since the uric acid, at high concentration, may be precipitated when combining with sodium ions (especially since the sodium ions concentration is relatively high in the plasma)[6].In the blood, (Table 3), it is slightly different regarding the K^+ ions. Here the K^+ ions level increases, while the other components show no difference from serum. The reason for this difference between blood and serum samples is due to the presence of the red blood cells in the blood sample. The contents of the RBC will be liberated into the plasma[7]. Also the metabolism inside the RBC affects the level of some blood constituents, (the clearest evidence of the continuation of its metabolism is the rapid decrease in the glucose level in a stored blood sample), it is found that in a stored blood sample for 30 minutes, 30% of the blood glucose will be lost.Also the activity of any cell leads to the liberation of K^+ ions, and this is evident during exercise, since there will be accumulation of what is called "Local Tissue Factors", and the potassium ions are one of these tissue factors. This liberation of K^+ ions might be due to the hyperactivity of the Na^+ -K^+ ATPase pump[7]. This will affect largely the concentration of the potassium ions, but the sodium ions will be so affected because its concentration is very high relative to K^+ ions[7].Another thing can be noted. The concentrations of the urea and cholesterol are not constant in the body, since the serum and blood samples are collected separately (at different times), and this is shown by the difference in the concentrations of these substances in the blood and serum samples at the zero time[8]. The uric acid is less variable, while we can see that the electrolytes are highly regulated in the body, since their concentrations are approximately constant all the time inside the body, and this is very important because it affects the excitability of all the cells of the body, including those of the heart.To keep the level of the blood or serum concentrations constant for a long time, we should use what is called the "preservatives"[9,10,11]. Surely, the conditions of the storage largely affect the constancy of the blood constituents. Some preservatives are used to keep some constituents of the blood constant since they may be destroyed by the enzymes present in the plasma. But, there is no single preservative for all the constituents of the blood. Even temperature affects the blood constituents, since it also affects the enzymes of the plasma and the stability of the constituent itself[12]. So, certain conditions may keep the concentration of a constituent constant not affected by time.How evert not all the constituents can be kept by the preservatives, so the analysis of these components must be done immediately when the blood sample is taken. The measurements of K^+ concentration. It cannot be kept by a preservative since the increase in the K^+ occurs due to the passage of the potassium ions found in the RBC to the plasma, so the only way in which we can keep the concentration of the potassium in the plasma constant is to prevent the passage of K^+ ions from the RBC to the plasma, and the way to do this is by separating the blood sample (to obtain the serum) immediately when the blood is collected[7].As a result, in the analysis of any sample we should take 3 important things in account to get right results and right diagnosis. The first is what sample we are analyzing, what constituent we measure, and lastly how much was the sample delayed before the analysis is done. All these 3 factors affect the diagnosis from the result of the sample analysis, especially for K^+ and uric acid measurement where specimen should be separated within 2 hours and stored as serum , stored at 4^oC.

Table1. Characteristics of the subjects who participated in the study

Characteristic	Value
Total Number	**56**
Male	**35**
Female	**21**
Weight (kg)	**65 ± 9.8**
Height (cm)	**171.6 ± 9.4**
Serum cholesterol (mg/dl)	**187.9 ± 28.6**
Blood urea (mg/dl)	**29.3 ± 4.6**
Serum uric acid (mg/dl)	**5.6 ± 0.8**
Serum potassium (mEq/L)	**3.9 ± 0.33**
Serum sodium (mEq/L)	**137 ± 5.6**
Serum chloride (mEq/L)	**99 ± 4.1**

Table 2. Effect of the storage, at 4°C, on the concentrations of TC, urea, uric acid, Na^+, K^+ and Cl^- in a serum sample (N=56)

Parameter	Storage time			
	0 time	1 day	2 days	6 days
Total cholesterol (mg/dl)	187.9 ± 28.6	189.6 ± 31.4	186.7 ± 26.4	185.2 ± 27.3
Urea (mg/dl)	29.3 ± 4.6	27.6 ± 5.2	25 ± 5.4	24.2 ± 4.6
Uric acid (mg/dl)	5.6 ± 0.8	5.4 ± 0.9	5.1 ± 1.0	4.8 ± 0.7
Sodium (mEq/L)	137 ± 5.6	140 ± 4.2	139 ± 3.2	140 ± 3.0
Potassium (mEq/L)	3.9 ± 0.33	3.8 ± 0.28	4.1 ± 0.27	4.0 ± 0.24
Chloride (mEq/L)	99 ± 4.1	100 ± 4.7	99 ± 4.4	97 ± 3.5

Table 3. Effect of the storage, at 4°C, on the concentrations of TC, urea, uric acid, Na^+, K^+ and Cl^- in plasma in contact with Red Cells (N=56).

Parameter	Storage time				
	0 time	2 hours	4 hours	6 hours	24 hours
Total cholesterol (mg/dl)	171 ± 29	173 ± 31	175 ± 28	172 ± 33	169 ± 32
Urea (mg/dl)	25 ± 4.3	24 ± 3.9	22 ± 3.6	21.7 ± 4.2	20 ± 4.3
Uric acid (mg/dl)	5.2 ± 0.6	4.8 ± 0.9	4.7 ± 1.1	4.5 ± 0.8	4.2 ± 1.1
Sodium (mEq/L)	140 ± 3.1	139 ± 2.9	138 ± 2.8	139 ± 3.2	138 ± 3.3
Potassium (mEq/L)	3.7 ± 0.24	3.85 ± 0.3	4.1 ± 0.27	4.4 ± 0.25	4.8 ± 0.25*
Chloride (mEq/L)	100 ± 4.1	98 ± 3.9	96 ± 4.2	97 ± 3.8	96 ± 3.7

*** $p < 0.05$**

References:

1. Burtis CA, Ashood ER. Tietz text book of clinical chemistry 2nd edn. Sanders company, advision of Horcount and company. Philadephia, London. 1994.
2. Zilva JF, Pannall RR. Clinical chemistry in diagnosis and treatment. 4th edn. Richarel Clay Ltd, Banngoy Suffolk. 1989.
3. Wirth WA and Thompson RL. The effect of various condition and substances on the results of laboratory procedures. Amer. J. Clin. Path. 43, 579-582.1965.
4. Wooten ID and Freemen H. Microanalysis in medical biochemistry. 16th edn. churchill living stone, Inc. Edinburgh, London and New York. 1982.

Murray RK, Granner DK, Mayes PA. Rodwell YW. Harper's Biochemistry. twenty – second edition. Beirut, Labanon. 1993.

5. Yamakita. J, Yamamoto T, Moriwaki Y, Takahashi S, et al,. Effect of urine storage on urinary uric acid concentrations. Ann – Clin – Biochem. 37 (pt3): 355-9 2000.
6. Gary GS, Barry MB. Fluid and electrolyte balance, Potassium. In: Fauci AS, Eugen E, Isselbacher KJ, Wilson JI, Martin. J.B, Kasper DI ,et al. Harrison's principles of internal medicine. 14th edn. Edited by McGraw-Hill company inc. Chapter 49:271-277. 1998.
7. Clausen T. Everts ME. Regulation of Na-k pump in skeletal muscle. Kidney int 35:1-13. 1989.
8. Rama Krishnan L, Reddy KS, Jaikhani BL. Measurement of cholesterol and triglycerides in dried serum and the effect of storage. Clin – Chem 47(6): 1113-5. 2001.

9. de – Wildt – Eggen. J, Schrijver, Johnna G, Bins, Mararten, Gulliksoon – Hans, et al. Storage of platelets in additive solution; effect of magnesium and/or potassium. Transfusion. 42(1):76-80. 2002.
10. Jones AW, Hylen L, Svensson E, Helander A. Storage of specimens at 4 degrees C or addition of sodium fluoride (1%) prevents formation of ethanol in urine inoculated with Candida albicans. J-Anal – Toxicol 23(5): 333-6. 1999.
11. Snabaitis AK, Shattoch MJ, Chamber DJ. Long- term myocarolial preservation: effects of hyperkalemia, sodium channel, and $Na/K^{+}/2Cl$ cotransport inhibition an extra cellular potassium accumulation during hypothermic storage. J-Thorac-Cardiovasc-Surg. 118(1): 123-34. 1990.
12. Hicks, M, Du ZY, Jansz P, Rainer S, et al. ATP sensitive potassium channel activation mimics the protective effect of ischaemic preconditioning in the rat isolated working heart after prolonged hypothermic storage. Clin – Exp – Pharmacol – Physicl-26(1):20-5. 1999.

تأثير الخزن على بعض مكونات الدم ومصل الدم

لمياء مصطفى النعمة ، جمال احمد عبدالباري ، عبدالقادر عبدالوهاب

الملخص :

لغرض فحص احتمالية خزن نموذج الدم او مصل الدم من ان يسبب تغيراً ببعض مكونات الدم ، تم تعين مستويات الكلولسترول الكلي ، جوهر البول ، حامض اليوريك ، الصوديوم ، البوتاسيوم و الكلوريد في نموذج بلازما الدم و مصل الدم خلال فترة خزن لمدة 2 ، 4 ، 6 ساعة و 1 ، 2 و 6 يوم . تم اختيار 56 شخصاً (35 من الذكور و 21 من الاناث) معدل اعمارهم 21 سنة (تتراوح من 19 – 23 سنة) . كانوا من غير المدخنين والذين هم ظاهرياً اصحاء وان معدل اوزانهم ضمن الحدود الطبيعية والذين لايعانون من مشاكل صحية ومن غير المتعاطين للادوية خلال الاسبوع الماضي من الدراسة، وقد تم قياس مستويات الكولسترول الكلي ، جوهر البول ، حامض اليوريك ، الصوديوم ، البوتاسيوم والكلوريد لهم في الاوقات صفر ، 2 ، 4 و 6 ساعة و 1 ، 2 ، 6 يوم . ان مضي الوقت او الخزن للدم قد اثر على تراكيز بعض مكونات الدم . ففي نموذج مصل الدم وخلال فترة الخزن البالغة 6 يوم . لم يؤثر الخزن على تراكيز الكولسترول الكلي ، الصوديوم ، البوتاسيوم والكلوريد وبقيت تراكيزهم تقريباً ثابتة بينما اثر الخزن على تراكيز جوهر البول وحامض اليوريك وسبب انخفاضاً غير معنوياً .

اما في نموذج من بلازما الدم فان تأثير الخزن لمدة 2 ، 4 ، 6 و 24 ساعة قد سبب ارتفاعاً معنوياً بمستوى البوتاسيوم وانخفاضاً غير معنوياً بمستويات جوهر البول وحامض اليوريك عندما حفض نموذج الدم بدرجة 4 ° م لمدة 24 ساعة . واستناداً لهذه النتائج ... نوصي بضرورة فصل مصل الدم عن كريات الدم الحمراء لان بقاء كريات الدم الحمراء لمدة 24 ساعة او اقل دون فصلها تؤدي الى حدوث تغيراً ببعض مكونات الدم .