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Original Research

Effect of Time Delay at Ambient Temperature on Serum Electrolytes and Comparison of Electrolyte Concentrations in Plasma and Serum

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Abstract

The present study was aimed at investigating the effect of time delay at ambient temperature on laboratory investigation of serum electrolytes and to compare the electrolytes concentrations in plasma and serum. The study was conducted by recruiting 32 healthy individuals of 22-35 years. Whole blood samples (6 mL) were collected from each individual and plasma and serum were separated. Serum was divided into four aliquots. One aliquot was analysed immediately after the separation (baseline value) and other three aliquots were kept for 1, 3 and 5 hours at ambient temperature (28-30 °C). The plasma and serum electrolytes; Na^+, K^+, Cl^- and iCa^{2+} concentrations were measured by ST-200 Plus Electrolyte Analyzer. There were no statistically significant differences in serum Na^+ concentrations between serum analysed immediately after separation (142.60±2.31 mmol/L) and serum kept for 1 hour at ambient temperature (142.63±2.27 mmol/L) (p>0.05). Similarly, no statistically significant differences were observed in serum Cl^- concentrations between serum analysed immediately after separation (101.27±1.46 mmol/L) and serum stored at ambient temperature for 1 hour (101.43±1.48 mmol/L) and 3 hours (101.55±1.39 mmol/L) (p>0.05). Comparison of baseline plasma and serum electrolyte concentrations demonstrated no statistically significant difference in Na^+ concentrations between plasma (142.75±2.23 mmol/L) and serum analysed immediately after separation (142.60±2.31 mmol/L) (p>0.05). However, a statistically significant difference was observed between plasma and serum K^+ concentrations, with plasma showing 4.09±0.31 mmol/L and serum analysed immediately after separation showing 4.21±0.31 mmol/L (p<0.05). Likewise, a statistically significant difference was observed between plasma and serum iCa^{2+} concentrations, where plasma showed 1.25±0.03 mmol/L and serum analysed immediately after separation showed 1.27±0.03 mmol/L (p<0.05). No statistically significant difference was observed between plasma (101.54±1.33 mmol/L) and serum analysed immediately after separation (101.27±1.46 mmol/L) in relation to Cl^- concentrations (p>0.05). Immediately separated plasma following blood collection can be suggested as the sample of choice for the determination of Na^+, K^+, Cl^-, and iCa^{2+} when multiple electrolyte parameters are analysed from a single sample.

Keywords: *Ambient temperature, Electrolytes, Plasma, Serum*

Introduction

Electrolytes are minerals that carry positive or negative electrical charges when dissolved in aqueous media. They exist as cations or anions and include acids, bases, and salts [1]. The major electrolytes present in the human body include sodium, potassium, chloride, calcium, phosphate, magnesium, bicarbonate, and sulfate [2]. These electrolytes are essential for maintaining normal physiological functions and play critical roles in acid–base balance, fluid balance, nerve impulse transmission, and muscle contraction.

Sodium is the predominant cation in extracellular fluid, whereas potassium is the principal intracellular cation. Chloride is the major extracellular anion. Sodium and potassium are important in maintaining osmotic pressure in extracellular and intracellular compartments, respectively. Potassium is also involved in cardiac muscle contraction and regulation of hydrogen ion concentration. Chloride contributes to maintaining electrical neutrality by moving passively with sodium and in exchange with bicarbonate. Furthermore, chloride plays an essential role in carbon dioxide transport through the chloride shift mechanism and contributes to oxygen exchange. Calcium is a vital structural component of bones and teeth and is involved in blood coagulation, cell division, endocrine regulation, and cardiac function [3,4].

Electrolyte imbalance may result from various conditions, including alcohol consumption, excessive sweating, cirrhosis, diabetes mellitus, kidney diseases, severe burns, trauma, eating disorders, hypertension, congestive heart failure, and thyroid and adrenal gland disorders [5,6]. Disturbances in electrolyte homeostasis can lead to severe and potentially life-threatening complications [2]. Potassium imbalance may adversely affect renal, cardiovascular, muscular, and neurological functions. Severe hypokalemia may result in respiratory compromise and paralysis [7,8]. Hypocalcemia can present with manifestations ranging from asymptomatic conditions to severe complications such as seizures [9,10]. Hypercalcemic crisis is a potentially life-threatening condition that may result in cardiac rhythm abnormalities, hypotension, renal failure, neurological impairment, and musculoskeletal complications including osteoporosis and bone pain [11,12]. Sodium imbalance affects nearly all body cells, particularly those of the central nervous system. Hypernatremia can result in cellular dehydration and brain cell shrinkage, whereas hyponatremia may lead to cerebral edema [13,14].

Numerous studies have investigated the association between serum electrolyte concentrations and diseases such as pancreatic disorders, kidney diseases, sickle cell disease, and COVID-19 [15–17]. However, relatively few studies have focused on the impact of pre-analytical factors on electrolyte determination in medical laboratories.

Accurate measurement of serum electrolyte concentrations is critical for appropriate clinical decision-making and patient management. Pre-analytical variables, including delays in sample processing and analysis, may affect the reliability of electrolyte measurements and consequently influence laboratory reporting.

Therefore, timely processing and analysis of blood samples are essential to ensure accurate electrolyte determination and minimize the risk of clinical misinterpretation arising from inaccurate laboratory results.

Delays in serum/plasma electrolyte analysis due to heavy laboratory workload or prolonged sample transportation from hospital wards and collection centers are common challenges encountered in many hospitals and laboratory settings, particularly in developing countries. Such delays may contribute to preanalytical variations that can potentially influence laboratory results. Although several studies have investigated preanalytical factors affecting electrolyte measurements [18-23], the effect of time delay at ambient temperature on plasma and serum electrolyte concentrations, specifically Na^+, K^+, Cl^-, and Ca^{2+} when requested together in a single laboratory test panel, has not been adequately investigated.

This issue is particularly relevant in tropical countries characterized by warm and humid climatic conditions, where delays in sample processing may have a greater impact on analyte stability. Therefore, understanding the influence of delayed sample processing at ambient temperature on electrolyte concentrations is important to ensure analytical accuracy and reliability in clinical laboratories.

The present study was designed to investigate the effect of delayed processing at ambient temperature on serum electrolyte measurements, specifically Na^+, K^+, Cl^-, and Ca^{2+} concentrations, and to compare electrolyte concentrations between plasma and serum samples. Plasma can be separated immediately following blood collection through centrifugation of anticoagulated blood, whereas serum preparation requires a clotting period before centrifugation. This difference in sample processing time may introduce preanalytical alterations that could influence electrolyte measurements. Therefore, the present study aimed to determine whether delayed serum separation contributes to significant changes in electrolyte concentrations compared with plasma and to evaluate whether differences exist between plasma and serum electrolyte measurements under routine laboratory conditions

Methods

Study design

This study was an analytical observational cross-sectional study with laboratory investigations.

Sample size calculation

The sample size was determined using a one-way analysis of variance (ANOVA) statistical power calculator, considering the study design and expected variability of electrolyte measurements. Potassium was used as the reference analyte for sample size estimation because it yielded the largest required sample size among the electrolytes evaluated, thereby ensuring adequate statistical power for all electrolytes included in the study.

The calculation was performed using the following parameters: number of groups (levels) = 5, maximum expected difference between

group means = 0.44, standard deviation = 0.50 (19), and statistical power = 80% (0.80).

The one-way ANOVA power calculation estimates the required sample size by incorporating the number of study groups, expected effect size derived from the maximum difference between means, variability within the data (standard deviation), and the desired statistical power. Among all electrolytes assessed, potassium generated the highest sample size requirement. Therefore, to ensure sufficient statistical power across all electrolyte analyses, a sample size of 32 samples per electrolyte parameter was selected for the study.

Study population

Participants were recruited using the convenient sampling method. Prior to study commencement, all participants were provided with an information sheet outlining the study details, including its objectives, implications, expected outcomes, and sample collection procedures. Written informed consent was obtained before participation. A self-administered questionnaire was used to collect socio-demographic information from the participants.

The study population included 32 healthy individuals (male and female) within 22-35 years. Individuals clinically diagnosed with dehydration, vomiting and other clinical manifestations due to imbalanced serum electrolytes, cirrhosis, diabetes mellitus, kidney diseases, trauma, eating disorders, hypertension, congestive heart failure, thyroid and adrenal gland disorders, pregnant and lactating women, people with burns, people with alcohol consumption, people use any medicine that causes alteration in serum electrolytes concentrations, individuals undergone surgeries and people doing severe exercises with excessive sweating were excluded.

Collection of blood samples

Venipuncture was performed by a well-experienced phlebotomist between 9:00 and 9:30 a.m. while adhering to standard safety precautions. No specific patient preparation was required prior to blood collection for serum/plasma electrolyte determination. A total of 6 mL of whole blood was collected from each participant and immediately divided into two aliquots by gentle transfer to avoid haemolysis. Of the collected blood, 1 mL was transferred into a lithium heparin-containing tube for plasma separation, while the remaining 5 mL was transferred into a plain tube without anticoagulant for serum separation.

Separation of plasma

The sample aliquot of 1 mL was centrifuged at 3000 rpm for 5 minutes to separate plasma. Plasma was analysed immediately after the separation to determine the baseline electrolytes concentration.

Separation of serum

Five milliliter blood sample was left undisturbed at room temperature for 30 minutes by keeping the plain tubes with blood in an upright position in a tube rack. After 30 minutes, the tubes were checked for clot formation. All the samples were observed clotted after 30 minutes. Then, the tubes were gently tapped in order to detach the

clot from the bottom of the tubes. Next, the clotted blood samples in plain tubes were centrifuged at 3000 rpm for 5 minutes. After the centrifugation, tubes were observed for haemolysis and serum was separated.

The effect of time-delay on serum electrolyte concentration

Each serum sample was divided into four aliquots each with 200 µL and transferred into four Eppendorf tubes which were properly labeled according to the respective serial numbers. All the Eppendorf tubes were capped tightly and placed in a plastic tube rack. Out of four serum aliquots, aliquot No: 1 was analysed for electrolytes immediately upon serum separation. Aliquot No: 2, 3 and 4 were kept for 1, 3 and 5 hours at ambient temperature (28-30 °C), respectively.

The concentrations of four serum electrolytes i.e., sodium (Na^+), potassium (K^+), chloride (Cl), and ionised calcium (iCa^{2+}) were measured using SENSA CORE ST-200 Plus semi-automated, microprocessor-controlled electrolyte analyzer (India). Direct Ion-selective Electrode (ISE) method was used to determine the serum electrolytes. Before the analysis, the analyzer was calibrated for the serum electrolytes. Following the calibration of the analyzer, two quality control samples (ST-200 (Pro/Plus/CL) reagent pack, India) were used to check the quality of the clinical chemistry assay samples in the analyzer. The plasma and serum samples were analysed after obtaining the results of the quality control samples.

Data analysis was done using IBM Statistical Package for Social Sciences (SPSS) software version 25.0. Paired sample t-test was used to compare the electrolyte concentrations in plasma and serum. One-way repeated measures ANOVA test was used to compare the mean values of electrolyte concentrations in serum samples which kept for 1, 3 and 5 hours at ambient temperature. The Dunnett t (2-sided) test was used to compare the electrolyte concentrations in serum analysed immediately after the separation with serum kept at ambient temperature for 1, 3 and 5 hours. In order to check the individual differences of mean serum electrolyte concentrations at different time intervals, the post-Hoc comparisons using the Tukey HSD (Honest Significant Difference) test was used.

Results

Comparison of electrolyte concentrations in plasma and serum

Table 1 shows the electrolyte concentrations of plasma and serum kept under the ambient temperature for different time intervals. The electrolyte concentrations of the 32 plasma samples vary as; Na^+; 138.10-145.80, K^+; 3.52-4.81, Cl^-; 98.40-104.00 and iCa^{2+}; 1.18-1.32 mmol/L. Similarly, baseline serum electrolyte concentrations vary as Na^+; 138.40-146.00, K^+; 3.65-4.95, Cl^-; 98.30-104.20 and iCa^{2+}; 1.21-1.35 mmol/L.

Though there was a reduction in baseline Na^+ concentrations in serum compared to plasma it was not statistically significant (*p>0.05*). The serum K^+ concentration was higher than the plasma and it was statistically significant (*p=0.000*). Even though the serum Cl^-

concentration was less than the plasma it was not statistically significant (*p>0.05*). In relation to iCa^{2+} concentration, there was a significant difference between baseline concentrations of serum and plasma (*p=0.006*). The iCa^{2+} concentration in serum was 0.02 mmol/L higher than plasma.

Effect of time-delay on serum sodium ion concentration

Table 1 indicates the Na+ concentrations given by serum samples; immediately after the separation and which kept for 1, 3 and 5 hours at ambient temperature.

Comparison of sodium ion concentrations in serum

Sodium ion concentrations were not same in the serum samples kept for 1, 3 and 5 hours at ambient temperature. According to the post-Hoc test there was a significant difference in Na^+ concentrations in serum kept for 1 and 3 hours (*p*=0.003). There was a reduction in serum Na^+ concentration at 3 hours compared to 1 hour and the difference was 0.39 mmol/L. There was a reduction in Na^+ concentration at 5 hours compared to 1 hour. The difference was 0.61 mmol/L and statistically significant (*p*=0.000). There was a reduction in serum Na^+ concentration at 5 hours compared to 3 hours and the difference was 0.22 mmol/L. However, it was not statistically significant (*p*>0.05).

According to the Dunnett t (2-sided) test, serum kept for 1 hour showed a higher Na^+ concentration compared to the serum analysed immediately after the separation, but it was not statistically significant (*p*>0.05). Three hour kept serum showed a lower Na^+ concentration compared to serum analysed immediately after the separation and the difference was 0.36 mmol/L. This difference was statistically significant (*p*=0.005). The same trend was observed between the serum analysed immediately after the separation and kept for 5 hours with the *p*=0.000. The difference of Na^+ concentration was 0.58 mmol/L.

Table 1 Electrolytes concentrations of plasma and serum kept under the ambient temperature for different time intervals.

Sample	Sodium	Potassium	Chloride	Ionised Calcium
Immediately upon plasma separation	142.75±2.23	4.09±0.31	101.54±1.33	1.25±0.03
Immediately upon serum separation	142.60±2.31	4.21±0.31	101.27±1.46	1.27±0.03
Serum after 1 hour at ambient temperature	142.63±2.27	4.24±0.30	101.43±1.48	1.24±0.03
Serum after 3 hours at ambient temperature	142.24±2.26	4.20±0.31	101.55±1.39	1.12±0.04
Serum after 5 hours at ambient temperature	142.02±2.15	4.20±0.33	101.72±1.42	1.16±0.08

Note: n = 32, SD = Standard Deviation, Electrolytes levels are given as Mean±SD in mmol/L

Effect of time-delay on serum potassium ion concentration

Table 1 shows K^+ concentrations given by serum samples, immediately after the separation and kept for 1, 3 and 5 hours at ambient temperature.

Comparison of potassium ion concentrations in serum

There were no statistically significant differences of K^+ concentrations in serum, immediately after the separation and kept for 1, 3 and 5 hours at ambient temperature (p>0.05).

Effect of time-delay on serum chloride ion concentration

The Cl^- concentrations given by serum samples, immediately after the separation and kept for 1, 3 and 5 hours at ambient temperature are given in Table 1.

Comparison of chloride ion concentrations in serum

The results showed that serum Cl^- concentrations were increased with time. However, there was no significant difference in Cl^- concentrations between 1 and 3 hours serum samples and 3 and 5 hours serum samples (p>0.05). However, there was a 0.29 mmol/L increase in Cl^- concentration at 5 hours compared to 1 hour and it was statistically significant (p=0.017). However, there were no significant differences in Cl^- concentrations in 1 and 3 hours kept serum samples compared to serum analysed immediately upon separation (p>0.05). However, there was a 0.45 mmol/L elevation in serum Cl^- concentration after 5 hours compared to serum of immediately analysed after the separation. This difference was statistically significant (p=0.002).

Effect of time-delay on serum ionised calcium concentration

The iCa^{2+} concentrations given by serum samples, immediately after the separation and kept for 1, 3 and 5 hours at ambient temperature are given in Table 1.

Comparison of ionised calcium concentrations in serum

The results showed that serum iCa^{2+} concentrations were decreased with time. There was a 0.05 mmol/L reduction in iCa^{2+} concentration at 3 hours compared to 1 hour and it was a statistically significant difference (p=0.000). The same trend was observed between 5 hours and 1 hour serum with the reduction of 0.08 mmol/L iCa^{2+} in 5 hours compared to 1 hour with the p = 0.000. Further, there was a 0.03 mmol/L reduction in iCa^{2+} concentration at 5 hours compared to 3 hours and it was a statistically significant difference (p=0.021).

According to the Dunnett t (2-sided) test, there were significant differences in iCa2+ concentration in 1, 3 and 5 hours kept serum compared to the serum analysed immediately after the separation. The 1 hour serum showed a lower iCa^{2+}concentration compared to serum of immediately analysed after the separation and it was a statistically significantly difference (p=*0.004*). There was a 0.08 mmol/L reduction in iCa^{2+} concentration in 3 hours kept serum compared to serum of immediately after the

separation and it was also a statistically significant difference (p=0.000). There was a 0.11 mmol/L reduction in iCa^{2+} concentration in 5 hours kept serum compared to the serum of immediately analysed after the separation and it was statistically significant (p=0.000).

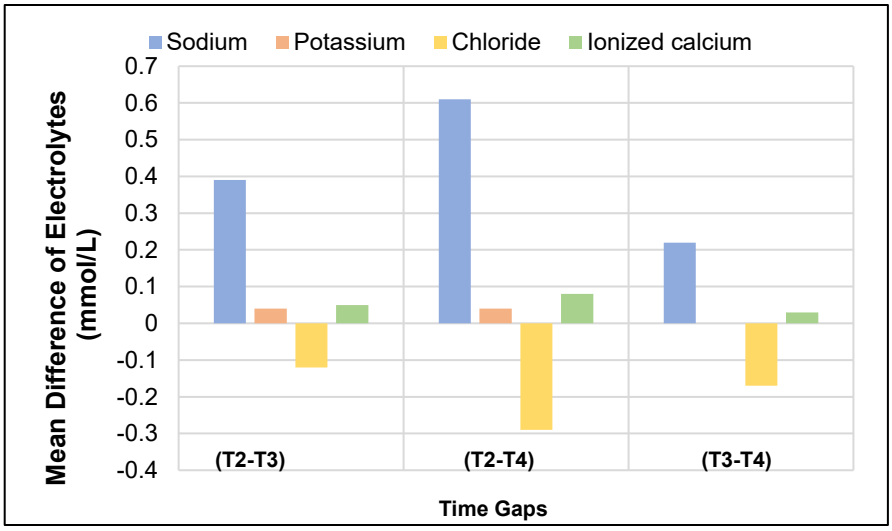

Figure 1 Comparison of mean difference of serum Na^+, K^+, Cl^- and iCa^{2+} at 1, 3 and 5 hours

Note: n=32, T_2 = Serum kept for 1 hour at ambient temperature, T_3 = Serum kept for 3 hours at ambient temperature, T_4 = Serum kept for 5 hours at ambient temperature

Mean differences in electrolyte concentrations

Comparison of all mean difference concentrations of serum Na^+, K^+, Cl^- and iCa^{2+} after 1, 3 and 5 hours i.e. mean serum electrolytes differences between serum kept for 1 hour and 3 hours, 1 hour and 5 hours and 3 hours and 5 hours are shown in Figure 1 whereas comparison of electrolytes concentration in serum analysed immediately after the separation with serum kept under the ambient temperature for 1, 3 and 5 hours ie., mean serum electrolytes differences between serum analysed immediately and 1 hour, serum analysed immediately and 3 hours and serum analysed immediately and 5 hours is given in Figure 2.

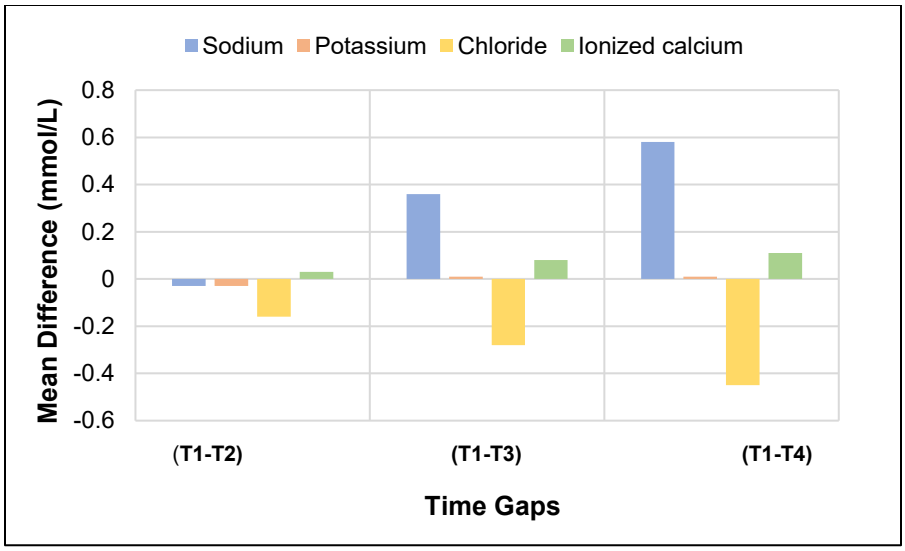

Figure 2 Comparison of serum electrolyte concentrations at different time points with the baseline concentrations

Note: n=32, T_1 = Serum immediately after the separation, T_2 = Serum kept for 1 hour at ambient temperature, T_3 = Serum kept for 3 hours at ambient temperature, T_4 = Serum kept for 5 hours at ambient temperature

Discussion

In the present study, we aimed to investigate electrolyte concentrations in plasma and serum to determine whether significant differences exist between the two sample types. Plasma separation can be performed immediately after blood collection, whereas serum separation requires a clotting period prior to centrifugation. During this delay, preanalytical changes may occur, potentially influencing electrolyte concentrations. Therefore, this study was conducted to evaluate whether the time required for serum separation contributes to significant alterations in electrolyte measurements compared with plasma.

The present study showed that stability of Na^+ altered after few hours of centrifugation. Serum Na^+ concentration decreased with time after 1, 3 and 5 hours. The study showed that significant differences of Na^+ concentrations between serum kept for 1 and 3 hours and 1 and 5 hours. There was also a significant difference in Na^+ concentration in 3 and 5 hour kept serum compared to serum analysed immediately after the separation. These findings are supported by the previous studies [8,24,25]. A pervious study showed that storing serum for a long time at 0-4 °C causes reduction of Na^+ concentrations. The Na^+ and K^+ in extracellular and intracellular fluid are maintained by Na^+/K^+ ATPase pump. ATP is required for active transportation of Na^+ and K^+ ions through cell membrane. If there is an inhibition of Na^+/K^+ ATPase pump, Na^+ is passed into the cells via concentration gradient, resulting in reduction of serum Na^+ concentrations [24]. Furthermore, it has been reported that serum Na^+ concentrations are significantly decreased in lysed blood samples as in haemolysis the serum sample is diluted as constituents of red blood cells come out. This results in reduction of serum Na^+ concentration

[18]. Another study showed that maximum acceptable delay for Na^+ in whole blood sample was 56 hours at room temperature and for serum samples for two weeks in the refrigerator [25].

In our study, baseline K^+ concentration in plasma and serum revealed that K^+ concentration in serum analysed immediately after separation is significantly higher than plasma. Further, findings of serum K^+ in aliquots kept at ambient temperature for 1, 3 and 5 hours showed that stability of serum K^+ is not altered significantly until 5 hours after the centrifugation. This finding is supported by the previous studies [24, 19]. It has been reported that K^+ was stable until 24 hours in both plasma and serum but K^+ concentration of serum was higher than plasma [20]. Razavi *et al.* (2010) showed that K^+ concentration in serum was higher than plasma in pediatric ICU patients [26]. Further, Hedayati *et al.* (2020) showed that maximum acceptable delay for K^+ in whole blood samples is 24 hours at room temperature [20]. If whole blood sample is stored too long at 0-4°C or kept more than 24 hours at room temperature led to increase in K^+ due to failure of Na^+/K^+ ATPase pump. Cool temperatures inhibit the Na^+/K^+ ATPase pumping action, and more K^+ is diffused from the erythrocytes lead to elevation of K^+ [24]. Another study showed that at the initial stage glycolysis is very fast, thus the serum K^+ concentration is decreased. When time passes, glucose in the serum is decreased and K^+ is passively diffused from cells and increases the concentration after long serum clot contact. This finding is in agreement with the results reported by Kalasker and Sudhamadhuri (2015) [19].

According to the present study, Cl^- concentration of plasma is higher than serum analysed immediately after the separation. However, it was not significantly different ($p>0.05$). Kalasker and Sudhamadhuri (2015) reported that serum clot contact time as a major source of pre-analytical variation in serum electrolytes [19]. Further, Hedayati *et al.* (2020) showed that maximum acceptable delay for Cl^- in whole blood and plasma/serum is 24 hours at room temperature [25].

We found that serum iCa^{2+} concentration was significantly higher than plasma iCa^{2+}. It has been reported that glucose in the red blood cells is converted into lactic acid in glycolysis. Glycolysis is increased when the blood sample is stored at room temperature for more than 15-30 minutes, subsequently decreasing the pH of the sample. As a result, the iCa^{2+} concentration is increased under the storage conditions mentioned. This observation is supported by the findings of Higgins (2020) [24, 27]. In our study, 30 minutes were taken for the serum separation, and this may be the reason for increased iCa^{2+} concentration in serum compared to plasma. McDonnell *et al.* (2002) has shown that iCa^{2+} concentration is decreased when the pH of serum is increased due to stronger binding of iCa^{2+} with proteins in the alkaline environment [21]. It has been reported that, a longer time gap between centrifugation and sample analysis causes a significant increase in pH and a statistically significant decrease in iCa^{2+} concentration. When the serum sample is exposed to the atmosphere, carbon dioxide is entered into the sample and pH is increased. This results in decrease in iCa^{2+} concentration

[28]. Further, Boyanton and Blick (2002) showed that maximum acceptable delay for Ca^{2+} in whole blood/plasma/serum is 24 hours at 4 °C and room temperature [20].

In addition to that, according to Baruah *et al*. (2014) falsely high serum electrolyte values could be obtained due to increased temperature up to 46 °C in summer, especially in tropical countries [22]. Moreover, the study revealed that the concentrations of Na^+, K^+ and Cl^- are increased with delay in processing of the serum samples after centrifugation.

The same study showed that stability of Na^+, K^+ and Cl^- was altered after few hours of centrifugation if there is a delay in analysis. The Na^+ and concentrations significantly increased at 3 and K^+ concentration increased significantly before 1 hour. A similar trend had been observed in the samples processed after 3 hours. However, the authors had been stated that all the samples had been kept with the lid opened during this period. The Na^+ had significantly increased at 3 and 5 hours. However, findings of this study contradict to the findings of similar studies. The possible reason for this difference may be the improper temperature maintenance in the laboratory leading to significant evaporation from the samples. Climatic conditions as well as opening sample containers under the fan for a few hours caused evaporation and falsely high serum electrolyte concentrations [22].

Donnelly *et al* (2009) showed that Na^+, K^+ and Cl^- are stable for 24 hours at room temperature, 4 °C and -20 °C [23]. Rahamtalla *et al.* (2017) has shown that effects of temperature and delay in separation of serum on K^+ and Na^+ concentrations. The results of the delayed whole blood samples had been compared with the immediately separated (within ½ h) whole blood samples. The centrifuged whole blood samples had been kept at room temperature for 2 and 4 hours in this study. The Na^+ and K^+ showed a reduction during these time intervals but comparison between ½ hour and after 2 and 4 hours showed that there are no statistically significant differences in these electrolytes [29]. The present findings too did not reveal any significant difference in baseline serum Na^+ concentrations and serum Na^+ concentrations at 1-hour intervals. Balbás *et al.* (2017) had investigated the stability of plasma electrolytes in Barricor and PST II tubes under different storage conditions. Samples were analysed at six time intervals since centrifugation i.e. 0, 3, 6, 9, 12 and 15 hours. The results showed statistically significant changes in Na^+ and Cl^- after 3 hours and in K^+ after 9 hours in both types of tubes at room temperature [30].

Another study showed that baseline K^+ concentration in serum was higher than plasma whereas the baseline Cl^- concentration in serum was lower than plasma. The same study reported that significant change was not observed in serum K+ kept for 48 hours at room temperature [31]. In the present study also there was no significant difference in baseline K^+ concentrations and K^+ concentration measured after 5 hours at the ambient temperature.

In this study, plasma samples separated immediately after collection demonstrated superior stability compared with serum. This is because the clotting process that may lead to

continued cellular metabolism and analyte leakage, resulting in artificially altered concentrations. In contrast, plasma obtained using anticoagulated tubes allows rapid separation of cellular components, minimising ongoing in vitro biochemical activity and thereby preserving the true in vivo electrolyte status more effectively.

A limitation of the present study is that, although serum electrolyte concentrations may be affected by contamination from intravenous fluids, the impact of this factor could not be evaluated.

Conclusions

Overall, plasma separated immediately after blood collection can be recommended as the sample of choice for the simultaneous determination of Na^+, K^+, Cl^-, and iCa^{2+} in laboratory testing, as prompt plasma separation minimises preanalytical variations and helps ensure the accuracy and reliability of electrolyte measurements.

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Ethical considerations

The study was conducted after obtaining ethical approval from the Ethical Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka (MLS/7/2022).

Declaration of conflicting interest

All authors declare that they have no conflicts of interest.

Authors' contributions

HFYCDF, WKGIW, and AMBP contributed to the conception of the research idea. The study was designed by AMBP and PD. Data acquisition was carried out by HFYCDF and WKGIW. Data analysis was performed by HFYCDF, WKGIW, and PD. The findings were interpreted by AMBP and PD. AMBP supervised the study and drafted the manuscript.

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