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RESEACH ARTICLE

INFLUENCE OF SERUM-CLOT CONTACT TIME ON RENAL FUNCTION PARAMETERS AMONG HEALTHY PARTICIPANTS IN SOKOTO, NIGERIA

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Abstract

Background: Clot contact time is an optimal time interval between sample collection and separation of serum from the clot. Delays in serum separation from red cells are often unavoidable in laboratory settings for various reasons. **Objectives:** To evaluate whether the stability of renal function parameters are affected in serum kept in contact with clot over time. **Methods:** This cross-sectional study involved fifty healthy adults enrolled to establish maximum acceptable delay time for renal function parameters. Venous blood specimens were collected and aliquoted into plain plastic tubes. The first clotted samples were centrifuged and the harvested sera serve as baseline sample (0 hr). The 2nd, 3rd, and 4th clotted samples were centrifuged after 4 hr, 8 hr and 12 hr respectively at room temperature for renal function tests. Data analysis was with Repeated Measures ANOVA Tool in Graph Pad InStat Software. **Results:** Serum kept in contact with clot for 4 hours at room temperature significantly increased in sodium and creatinine values. Increase in urea and potassium with decrease in chloride ion within 4 hours was also observed compared to 0 hour. Bicarbonate concentration increased significantly within 8 hours compared to the base line value and decreases after 8 hours. **Conclusion:** This study suggests that for optimal clinical utility, serum specimens should be separated immediately from clots for renal function test. However, the acceptable delay period for potassium, chloride, bicarbonate and urea is within 4 hours at room temperature.

Key words: clot contact, serum, temperature, Sokoto

INTRODUCTION

Clot contact time refers to optimum time interval between sample collection and separation of serum from the clot (Rashmi *et al*., 2014). Prolong contact of serum with clot cause pre-analytical variation, the optimum time interval between sample collection and separation of serum from clot should be long enough to allow complete clot formation. However, it should be shorter than the time in which a significant change in test result is induced by serum–clot contact (Zhang *et al*., 1998).

A number of factors, primarily pre-analytical, analytical and normal biological variations affect the accuracy of test results (Ahmed, 2010). Imprecision, or reproducibility error, may however be due to both physiological and analytical factors (Ahmed *et al*., 2007).

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During pre-analytical phase, the time interval between blood collection and sample analysis processing is a critical stage which is an error prone area and also the bottleneck in the turnaround time of the laboratory. Therefore, the two important time delay processes that occur in this phase is clot contact time and centrifugation delay (Rashmi *et al*., 2014).

Research has shown that serum–clot contact interferes with at least twenty-five (25) analytes during the analytical and pre-analytical phase. These significantly show changes in potassium at 2 hrs, chloride at 8 hrs while other tests are stable for up to 48 hrs (Laessig *et al*., 1976). Also increase in potassium ion levels at 4 hours of serum clot under room temperature has been reported by Ono *et al*. (1981). The science behind the increase in potassium ion could be as a result of K+ leaking from red cells in delayed serum-clot contact. Therefore, for stability of some biochemical analytes, it is reputable to separate the serum clot within 6 hours for reliable concentrations outcome (Zhang *et al*., 1998).

Urea is the major nitrogen containing metabolic product of protein catabolism in humans, accounting for more than 75% of the non-protein nitrogen eventually excreted (Burtis *et al*., 2008). Catabolism of proteins and amino acids results in the formation of urea, which is predominantly cleared from the body by the kidneys. Consequently, kidney disease is associated with accumulation of urea in blood. An increase in plasma urea concentration characterizes the uremic (azotemic) state (Burtis *et al*., 2008).

During the process of protein catabolism, amino acid nitrogen is converted to urea in the liver by the action of the so-called urea cycle enzymes. More than 90% of urea is excreted through the kidneys, with losses through the gastrointestinal tract and skin accounting for most of the remaining minor fraction.

Zhang *et al*. (1998) and Tanner *et al*. (2008) found urea concentration to increase significantly at clot-contact time 3 hrs and 6 hrs with a subsequent decrease after 24 hrs. Similarly, Chu and Macleod (1986) also reported a decrease in the concentration of urea.

Creatinine is a nitrogenous product which has a molecular weight of 113 Da, is produced from the metabolism of creatine in the skeletal muscles (Ochei and Kolhatkar, 2007; Bishop *et al*., 2010). This reaction is catalyzed by creatine kinase and is the first source of metabolic fuel used in muscle contraction (Bishop *et al*., 2010). Creatine phosphate and ADP in the presence of creatine kinase react to give creatinine and ATP. The creatine formed is nonenzymatically converted to creatinine (Bishop *et al*., 2010). The formation of creatinine is constant and has direct relationship to muscle mass. For this reason, it varies with age and gender (Ochei and Kolhatkar, 2007).

According to Chu and Macleod (1986) and Rehack and Chiang (1988) the creatinine concentration increases slightly after 6 hrs and Zhang *et al*. (1998) reported that creatinine is stable for up to 24 hrs.

Due to power instability and storage inadequacy, in order to mimic the ISO 15189 standard in our settings this study will be of immense relevance for reliable outcome of test results with improved precision and accuracy for diagnosis.

This study aims to mimic the ISO 15189 standards within our setting, acknowledging challenges such as power instability and limited storage facilities. Also, it seeks to provide valuable insights into establishing acceptable delay times when immediate sample processing is not feasible, ultimately enhancing the precision and accuracy of diagnostic test results. By identifying acceptable serum-clot contact times, this study will broaden our understanding of pre-analytical sources of error in laboratory workflows and contribute to improving the quality of laboratory management systems.

MATERIALS AND METHODS

Study Area

This study was carried out in Sokoto, North-Western Nigeria.

Study Population

Fifty (50) apparently healthy adults (18-53 years) within Sokoto metropolis were randomly selected for the study to establish maximum acceptable delay time before harvesting of serum for renal function test.

Inclusion Criteria

Apparently healthy adults of Sokoto resident were recruited for the study.

Exclusion Criteria

Subjects with recent blood transfusion (within 120 days) sickle cell disease, sign of jaundice, cigarette smoking, human immunodeficiency virus and acquired immune deficiency syndrome (HIV/AIDS) and other systemic

disorders such as systemic hypertension and diabetes mellitus were excluded from the study.

Informed Consent

The relevance and benefits of the study was explained to all enrolled individuals and related information about the study was discussed and that participation was entirely voluntary, with the right to withdraw at any time without penalty. Participants were not induced but appreciated with incentive and thereafter, the participants consent was obtained.

Ethical Considerations

The study was approved by the Ethics and Research Committee of State Ministry of Health, Sokoto (SKHREC/085/18).

Sample Size

The resource equation (Araoye *et al*., 2004) was used to calculate minimum sample size as 384. Due to financial constrain only 200 aliquots from 50 specimens were used for the study.

Study Design

This was a cross-sectional study design involving fifty healthy adults enrolled to establish maximum acceptable delay time before harvesting of serum for renal function parameters. The research sample collection was carried out within 5 days by recruiting 10 subjects per day. Venous blood specimen from each participant was collected into di-potassium ethylenediaminetetraacetic acid and 4 plain tubes (for 0hr, 4hrs, 8hrs and 12hrs). The anticoagulant treated blood was used as incentive for participants to know their genotype and HIV status. The blood collected in plain tubes were allowed to clot. The first clotted samples were centrifuged and the sera harvested served as baseline sample (0 hr). The 2^{nd}, 3^{rd}, and 4^{th} clotted samples were centrifuged after 4 hr, 8 hr and 12 hr respectively at room temperature (25^{o}C) which was monitored using a laboratory thermometer. All clotted samples at different time interval were centrifuged at 3000 rpm for 5 min in 25^{o}C. The harvested sera were used for Na^{+}, K^{+}, Cl^{-}, HCO_3^{-}, urea and creatinine estimations.

Repeated measures Analysis of Variance was used to determine any significant difference across different time intervals against the baseline analysis due to serum-clot contacts.

Sampling Techniques
Subject Selection
Apparently healthy adult individuals residing in Sokoto metropolis were selected by convenience approach for the study following informed consent with the aid of close ended questionnaire.

Blood Sample Collection and Processing

Blood sample was collected by venipuncture from the anterior cubital fossa. Six (6) mL of blood was drawn slowly and aseptically from each subject into a syringe. The blood specimen was aliquoted into dry plain bottles in four aliquots labelled 0 hr, 4 hrs, 8 hrs and 12 hrs. Following blood clotting, one of the clotted samples was centrifuged and the sera harvested serve as baseline sample (0 hr). The remaining clotted samples were observed after 4 hr, 8 hr and 12 hr at room temperature. All clotted samples at different time interval were centrifuged at 3000 rpm for 5 min. The harvested sera were analyzed for renal function parameters (Na^{+}, K^{+}, Cl^{-}, HCO_3^{-}, urea and creatinine) immediately at the scheduled times at the rate of 10 samples daily for the period of 5 days.

Analytical Methods

Estimation of Sodium, Potassium and Chloride Ions Activity

The estimation of sodium, potassium and chloride ions activity was performed using the method described by (Otto *et al*., 1985). This is a potentiometric method which directly measures change in the potential difference as compared to reference electrode due to the activity of free ions when in contact with electrode at constant current and resistance. The Ion Selective Electrode (Electrolyte analyzer, GE-300, China), machine was used.

Estimation of Serum Bicarbonate Ion Concentration

Bicarbonate concentrations were estimated using the method of Modified Van Slyke's (Gyory *et al*., 1997). This is a titration method, where serum is mixed with 0.01 N hydrochloric acid (HCl) in the presence of neutral red as an indicator, a decrease in acidity due to bicarbonate present in the sample is measured by titration against 0.01

N sodium hydroxide (NaOH). The end-point value was picked by the appearance of a yellow colour change. The equation Bicarbonate Conc. (mmol/L) = (1- titre) x 100 was used to calculate bicarbonate concentration.

Estimation of Serum Urea Concentration

The diacetyl monoxime method described by Marsh *et al*. (1965) was used for the determination of urea concentration.

Estimation of serum creatinine concentration

The method of Jaffe was employed as described by Bousnes and Taussky *et al*. (1975) as creatinine reacts in alkaline medium, with picric acid giving a yellowish-red colour.

Data analysis

The comparison of multiple variances of serum clot-contact at 0 hr, 4 hrs, 8 hrs and 12 hrs were applied using one-way repeated measures ANOVA test, assuming 95% confidence interval and significant parameters were analyzed with Bonferroni post-hoc test for mean separation. P-values less than 0.05 were considered to be statistically significant. Data analysis was carried out with GraphPad InStat3 version 3.02.

RESULTS

Electrolytes composition of serum clot contacts at different time interval

One-way repeated measure ANOVA was run and the result for Mauchly's test showed assumptions violation but corrected using Greenhouse-Geisser which showed a significant difference in the renal function parameters between clot-contact time (0 hr, 4 hrs, 8 hrs and 12 hrs). Bonferroni Post-hoc analysis revealed that sodium and potassium ion concentrations at 0 hr were significantly lower (p=0.001) than the concentrations at 8 hrs, and 12 hrs. When serum clot-contact of Na^+ concentration at 0 hr was compared after 4 hrs, it was not statistically significant but slight increase was noticed while potassium increases significantly (p =0.001). At 4 hrs, Na^+ concentration when compared after 8 hrs increased significantly (p =0.001) but then it decreases slightly after 12 hrs with no significant difference (p= 0.056). Conversely, K^+ concentration revealed non-significant (p=0.063) increase at 4 hr compared to 8 hrs serum clot contact but after 12 hrs the concentration of K^+ significantly increased.

The results of chloride ion concentration at 0 hr were reduced significantly (p=0.001) as compared to 4 hrs, 8 hrs, and 12 hrs. Serum bicarbonate concentration after 4 hrs and 8 hrs from baseline (0 hrs) was noticed to increase significantly (p=0.001), at 12 hrs bicarbonate concentration significantly (p =0.001) decreases when compared to 4 hrs and 8 hrs but did not differ significantly at 4 hrs when compared to 8 hrs. Serum concentration for urea levels at baseline (0 hr) decreases significantly (p=0.001) when compared with 4 hrs, 8 hrs and 12 hrs. The serum urea concentration at 4 hrs clot contact did not differ significantly (p =1.000) when compared after 8 hrs and 12 hrs clot-contact. A slight increase in creatinine concentration was noticed after 4 hrs, but not statistically significant (p=1.000) compared to baseline (0 hr). For 4 hrs clot-contact, as compared to 8 hrs and 12 hrs a significant increase (p= 0.001) was observed (Figure 1).

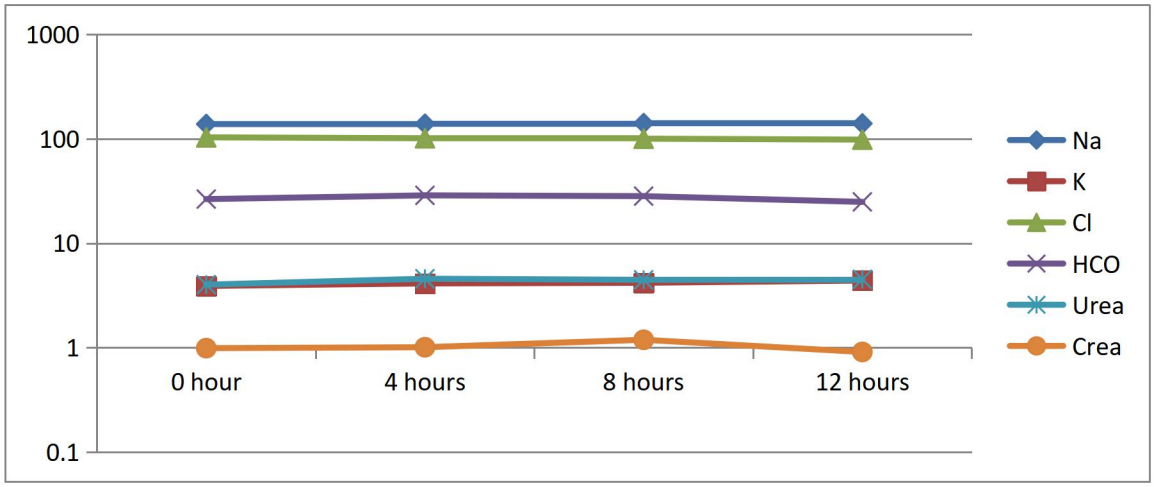

Figure 1: Changes in Kidneys Functions Values of Serum Clot-Contact Time

Table 1: Changes in Electrolyte Values of Serum Clot-Contact Time

		Parameters (mmol/L)			
Hours	**n**	**Sodium**	**Potassium**	**Chloride**	**Bicarbonate**
0 hour	50	137.96±1.54	3.90±0.25	103.26±2.06	26.42±2.37
4 hours	50	138.80±1.56	4.12±0.34	101.46±2.01	28.68±3.00
8 hours	50	140.68±2.68	4.18±0.35	100.40±2.24	28.20±3.10
12 hours	50	139.62±2.62	4.39±0.37	98.78±2.13	24.84±2.76
F-value		15.11	54.06	107.15	54.76
p-value		0.0001	0.0001	0.0010	0.0010
Post hoc					
0 hour vs 4 hours		0.076	0.001	0.001	0.001
0 hour vs 8 hours		0.001	0.001	0.001	0.001
0 hour vs 12 hours		0.001	0.001	0.001	0.001
4 hour vs 8 hours		0.001	0.063	0.001	1.000
4 hour vs 12 hours		0.056	0.001	0.001	0.001
8 hour vs 12 hours		1. 000	0.001	0.001	0.001

Values are presented as mean± SD. n=number of sample representation per time interval

Table 2: Effect of clot-contact time on urea and creatinine concentrations

		Parameters (mmol/L)	
Hours	**n**	**Urea**	**Creatinine**
0 hour	50	4.01±1.03	0.99±0.16
4 hours	50	4.57±1.16	1.01±0.16
8 hours	50	4.46±1.07	1.19±0.18
12 hours	50	4.51±1.21	0.91±0.22
F-value		9.33	62.19
p-value		0.0010	0.0001
Post hoc			
0 hour vs 4 hours		0.001	0.229
0 hour vs 8 hours		0.010	0.001
0-hour vs 12 hours		0.001	0.010
4 hour vs 8 hours		1.000	0.001
4 hour vs 12 hours		1.000	0.001
8 hour vs 12 hours		1.000	0.001

Values are presented as mean± SD.

DISCUSSION

In this study there was no statistically significant change in sodium ion concentration at 4 hrs of serum clot-contact, but there was an increase in the concentration of sodium which was statistically significant after 8 hrs and 12 hrs when compared to the base line (0 hr). The findings in this study are in agreement with (Ono *et al*., 1981) and (Chu and MacLeod, 1986). Contrastingly, the studies of Laessig *et al*. (1976), Rehack and Chiang (1988) and Heins *et al*. (1995) reports were not in agreement possibly due to active transport condition of sodium ion across the cell membrane. Other factors such storage temperature, processing may be attributed. For example, Heins *et al*. (1995) reported sodium stability at room temperature after 3 days while in the current changes was observed after 8 hours. Apparently, their study was conducted in Germany, which might have lower room temperature compared to the current study site.

Significant increase in potassium ion concentration at 4 hrs, 8 hrs, and 12 hrs compared with 0 hr was in line with the findings of (Laessig *et al*., 1976; Rehack and Chiang, 1988; Heins *et al*., 1995; Zhang *et al*., 1998; Tanner *et al*.,

2008; Oddoze *et al*., 2012), but were not in tandem with the finding of (Ono *et al*., 1981). The change of potassium may be attributed to the net effect of glycolysis, which moves potassium into cells, and passive diffusion, which allows potassium to diffuse out of the cells (Chen and Lui, 2022). This result shows that at room temperature, passive diffusion was dominant, allowing potassium to increase due to slower rate of glycolysis.

The decrease in chloride ion concentration in this research within 4 hrs compared to base line (0 hr) agrees with the studies of (Laessig *et al*., 1976; Chu and MacLeod, 1986; Heins *et al*., 1995). It was in contrast from the finding of (Rehack and Chiang, 1988; Zhang *et al*., 1998) that there was no statistically significant effect of serum clot contact time on chloride ion concentration kept at room temperature. The reason for the divergent finding is not known.

Bicarbonate ion was found to be increasing significantly as the serum clot-contact time increases within 8 hrs. Also, significant decrease in the concentration of bicarbonate after 12 hrs compared to 0 hr was observed. The finding was in line with that of (Zhang *et al*., 1998).

Previous studies are contradictory regarding the stability of urea and creatinine concentration during serum clot contact. For urea, (Zhang *et al*., 1998; Tanner *et al*., 2008) found significant increase whereas (Chu and MacLeod, 1986) reported a decrease in the concentration of urea. We observed an increase with significant difference across the timing points compared to baseline. This increase could be due to the facilitated diffusion of urea across the red blood cell membrane out of the cell (Brahm 1983; Brahm, 2013).

The finding of Zhang *et al*. (1998), Chu and MacLeod (1986) and Rehack and Chiang (1988) agrees with a slight increase in creatinine concentration noticed in this study within 4 hrs which did not show significant difference compared to baseline. This was significant after 8 hrs and 12 hrs clot contact compared to 4 hrs. The finding was in conflict with the findings of Laessig *et al*. (1976), Ono *et al*. (1981), Heins *et al*. (1995), Shepherd *et al*. (2007) that reported that creatinine is stable for up to 24 hrs. The transport of creatinine across the red blood cell membrane by passive diffusion was implicated as likely reason for the slight increase of creatinine (Buzdygon and Zydney, 1989).

Conclusion

This study suggests that for optimum clinical utility, serum specimens for should be separated immediately from clots for renal function test. However, the acceptable delay period for potassium, chloride, bicarbonate and urea is within 4 hours at room temperature.

Limitation of The Study

The study did not included large sample size in the study design.

Recommendations

Based on the findings from this study it is recommended that further work should be design to validate this findings in our community, taken into consideration the timing before 4 hrs. In the same vein, the inclusion of pathologic specimen is recommended for comparative studies which may be helpful in clinical laboratories.

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Conflict of Interest

The authors have declared no competing interest.

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This study did not receive any funding

Authors' Contribution

JI, conceived the study, wrote the manuscripts and analyzed the data, MU, carried-out the research work, YMH, supervised the field work. AHL, MAH, AR, MRI, YBM, AFZ, HSY, MI and HHD did the literature review, designed the study and reviewed the. All authors reviewed and approved the manuscript.

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