ejpmr, 2018,5(8), 356-361

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

INFLUENCE OF HEPARIN ON ELECTROLYTES ANALYSED ON ELECTROLYTE ANALYSER IN AN EMERGENCY BIOCHEMISTRY LABORATORY OF PGIMS ROHTAK – A COMPARATIVE STUDY WITH SERUM

Sabiha Naz*[1], Kiran Chugh[2] and Isha Malik[3]

[1]Demonstrator, Department of Biochemistry, Pt. B. D. Sharma Postgraduate Institute of Medical Sciences, Rohtak (Haryana).

[2]Professor, Department of Biochemistry, Pt. B. D. Sharma Postgraduate Institute of Medical Sciences, Rohtak (Haryana).

[3]Associate Professor, Department of Biochemistry, Pt. B. D. Sharma Postgraduate Institute of Medical Sciences, Rohtak (Haryana).

***Corresponding Author: Sabiha Naz**

Department of Biochemistry, Pt.B.D. Sharma Postgraduate Institute of Medical Sciences, Rohtak (Haryana).

Article Received on 11/06/2018 **Article Revised on 01/07/2018** **Article Accepted on 22/07/2018**

ABSTRACT

Background: Serum electrolytes (Sodium and Potassium) are conventionally measured in all critical patients of emergency department, patients receiving fluid therapy and those admitted to intensive care units. The electrolytes concentration can be measured by an electrolyte analyser in heparinised blood as well as in serum. Our present study is designed in order to find out the effect of heparin on electrolytes (sodium and potassium) in heparinised blood and to compare the heparinised blood electrolyte measurements and serum electrolyte measurements in same patient's sample. **Methods:** This is a prospective observational study conducted in the emergency laboratory of Biochemistry Department at Pt.B.D.Sharma PGIMS, Rohtak. We obtained coupled samples, one heparinised blood sample and other venous sample (for serum) from 150 patients admitted in the accidental and emergency department, intensive care units and different wards of Pt.B.D.Sharma PGIMS, Rohtak. We analyzed both of them on **Eschweiler Combiline Electrolyte Analyser** based on Direct ISE Method. The results obtained were uploaded to MS excel sheets and further divided into 3 groups viz **Group I** (having a very low variation in electrolyte values between heparinised blood and serum), **Group II** (having moderate variation in electrolyte values between heparinised blood and serum), and **Group III** (having high variation in electrolyte values between heparinised blood and serum).Statistical analysis was done using students **unpaired 't'** test. **Results:** In **Group I**, the mean sodium value in heparinised blood was 136.28 ±7.39 mmol/ L and mean sodium value in serum was 136.88 ±7.42 mmol/L(***p value>0.05***). In **Group II** ,the mean sodium value in heparinised blood was 134.01 ±7.72 mmol/ L and mean sodium value in serum was 136.93 ±7.62 mmol/L**(*p value<0.05*)** and In **Group III**, the mean sodium value in heparinised blood was 133.92 ±7.21 mmol/ L and mean sodium value in serum was 143.12 ±7.39 mmol/L**(*p value<0.001*)**. Similarly, In **Group I**, the mean potassium value in heparinised blood was 4.22 ±0.85 mmol/ L and mean potassium value in serum was 4.29 ±0.83 mmol/L (***p value>0.05***). In **Group II**, the mean potassium value in heparinised blood was 4.21 ±0.91 mmol/ L and mean potassium value in serum was 4.58 ±0.85 mmol/L**(*p value<0.05*)** and In Group III ,the mean potassium value in heparinised blood was 3.69 ±0.93 mmol/ L and mean potassium value in serum was 4.65 ±0.90 mmol/L**(*p value<0.001*)**. **Conclusion:** We concluded that serum electrolytes values in heparinised blood and serum were having statistical insignificant **(p>0.05)** difference in Group I while we obtained statistical significant **(p<0.05)** as well as statistical highly significant **(p<0.001)** difference in Group II and Group III respectively. These above stated significant differences may be due to improper dilution of blood sample with heparin. If heparin is used in proper quantity (i.e 50-80 IU/ml vol. of blood) correct and comparable results may be obtained on which critical clinical decisions can be made by trusting the electrolytes values obtained through heparinised blood testing on electrolyte analyser.

KEYWORDS: Electrolytes, Heparin, Sodium and Potassium.

INTRODUCTION

Determination of the serum electrolytes is one of the most frequent tests performed in laboratory medicine, both in central laboratories and at the point of care (causality). It is well known that rapid provision of blood measurements, particularly blood gases and electrolytes may translate into improved clinical outcomes. Studies have shown that point of care testing carries advantages of providing reduced therapeutic turnaround time, shorter clinical decision time, rapid availability of data and minimized pre- and post- analytic testing errors.[1]

Preanalytical errors are said to be the reason for up to 75 %[2] of all errors in laboratory medicine. The diagnostic consequences depend on the magnitude of the pre analytical error. The blood gas collection with liquid heparin in point of care or laboratory testing is one of the sources of pre analytical errors.[2-4] When heparin is added to whole blood it forms a complex with anti thrombin III, and the blood's ability to coagulate is inhibited. Heparin also binds all positive ions in blood. To eliminate this effect on potassium and sodium, some sampling devices also come with electrolyte- balanced heparin, which compensates for the binding effect within the normal range of the electrolytes. Using Li-heparin eliminates the adverse effect of Na or K- heparin on measurement of these electrolytes and Li-heparin is better choice for plasma biochemical analyses.[5] As **Figure. 1** shows, an addition of 0.05 mL liquid heparin to 1 mL whole-blood sample (Hct 45 %) will dilute the plasma phase by 10 %. Since the electrolyte parameters are determined in serum, the concentrations of these parameters will decrease accordingly.[6]

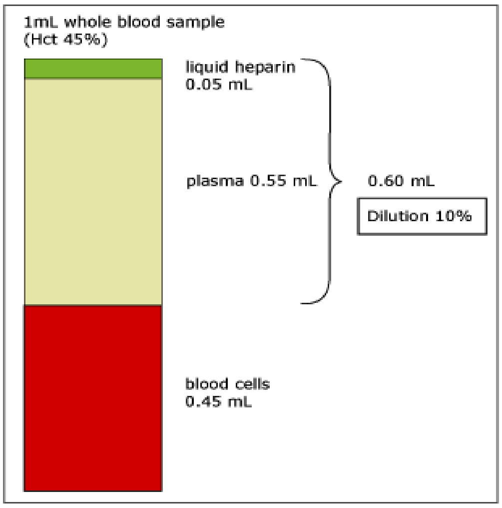

Figure 1: Dilution of Whole blood with Heparin.[6]

Routinely electrolytes are measured using auto analyzer, but nowadays Electrolyte analyzer facilitates measurement of serum electrolyte by **DIRECT ISE** but generally the later measurement is rarely trusted by the clinicians. So routinely, we have observed at our institute clinicians send the samples in liquid heparin syringes along with separate sample for electrolyte measurement in plain vacutainers. This is more time consuming and wastage of reagents.

With this background we designed a study to observe the effect of heparin on electrolytes (Sodium and Potassium) in heparinised blood which are compared with serum on the same electrolyte analyser.

MATERIALS AND METHODS

This is a prospective observational study which was conducted in the emergency laboratory of Biochemistry Department at Pt.B.D.Sharma PGIMS, Rohtak. We included newborn babies and adult patients (both gender), 18 years or above admitted to the accidental and emergency department, intensive care units and different wards who have arterial and central venous access in situ (under normal and pathological conditions).

150 Coupled Samples (one heparinised blood sample +one plain blood sample for serum) were obtained from 150 patients following standard ethical principles .Samples were collected by trained residents. Samples (2ml each) were collected both in heparinised syringe and plain vacutainers (red top) of the same patient simultaneously. Samples with inadequate volume and hemolysed were excluded from the study. Both heparinised blood samples and serum samples were analyzed for electrolytes (Sodium and Potassium) on **Eschweiler Combiline Electrolyte Analyser** based on Direct ISE Method. In the direct ISE method, the electrode surface contacts a complete undiluted blood sample.[7] Daily internal quality control was done on electrolyte analyzer to perform accurate testing. Along with this **EQAS** (External Quality Assurance System) testing was also performed every month to maintain quality of results.

STATISTICAL ANALYSIS

Results were recorded in tabular form in MS EXCEL Sheets. Further, results are classified into 3 groups viz **Group I** (having a very low variation in electrolyte values between heparinised blood and serum), **Group II** (having moderate variation in electrolyte values between heparinised blood and serum), and **Group III** (having high variation in electrolyte values between heparinised blood and serum). Statistical analysis was done by using student's **unpaired 't'** test. Level of significance was set as p value:-

$p > 0.05$ – not significant
$p < 0.05$ – significant
$p < 0.001$ – highly significant.

RESULTS

In our study, we had all age groups (new born babies and adults). Patients suffering from pathological conditions like myocardial infarction and renal failure were included. The other conditions like Chronic obstructive pulmonary disease, shock and pulmonary embolism, respiratory distress syndrome, poisoning, snake bite, end stage renal disease, intracerebral hemorrhage, diabetes mellitus and hypertension, spinal cord injury, diabetic keto acidosis were also included in this study.

Table No. 1: Comparison of Serum Electrolytes (Sodium and Potassium) in heparinised blood and serum analyzed on electrolyte analyser of Group I patients.

S. No.	Parameters	(Mean ±SD) in Heparinised blood (N=50)	(Mean ±SD) in Serum (N=50)	P value
1	Sodium (mmol/L)	136.28±7.39	136.88±7.42	>0.05 **(NS)**
2	Potassium(mmol/L)	4.22±0.85	4.29±0.83	>0.05 **(NS)**

(Note: p value >0.05 stands for non significant, p value <0.05* for statistically significant and p value <0.001** stands for highly significant).

Table No. 2: Comparison of Serum Electrolytes (Sodium and Potassium) in heparinised blood and serum analyzed on electrolyte analyser of Group II patients.

S. No.	Parameters	(Mean ±SD) in Heparinised blood (N=50)	(Mean ±SD) in Serum (N=50)	P value
1	Sodium (mmol/L)	134.01±7.72	136.93±7.62	<0.05* **(SS)**
2	Potassium(mmol/L)	4.21±0.91	4.58±0.85	<0.05* (SS)

(Note: p value >0.05 stands for non significant, p value <0.05* for statistically significant and p value <0.001** stands for highly significant).

Table No. 3: Comparison of Serum Electrolytes (Sodium and Potassium) in heparinised blood and serum analyzed on electrolyte analyser of Group III patients.

S. No.	Parameters	(Mean ±SD) in Heparinised blood (N=50)	(Mean ±SD) in Serum (N=50)	P value
1	Sodium (mmol/L)	133.92±7.21	143.12±7.39	<0.001** **(HS)**
2	Potassium(mmol/L)	3.69±0.93	4.65±0.90	<0.001** **(HS)**

(Note: p value >0.05 stands for non significant, p value <0.05* for statistically significant and p value <0.001** stands for highly significant).

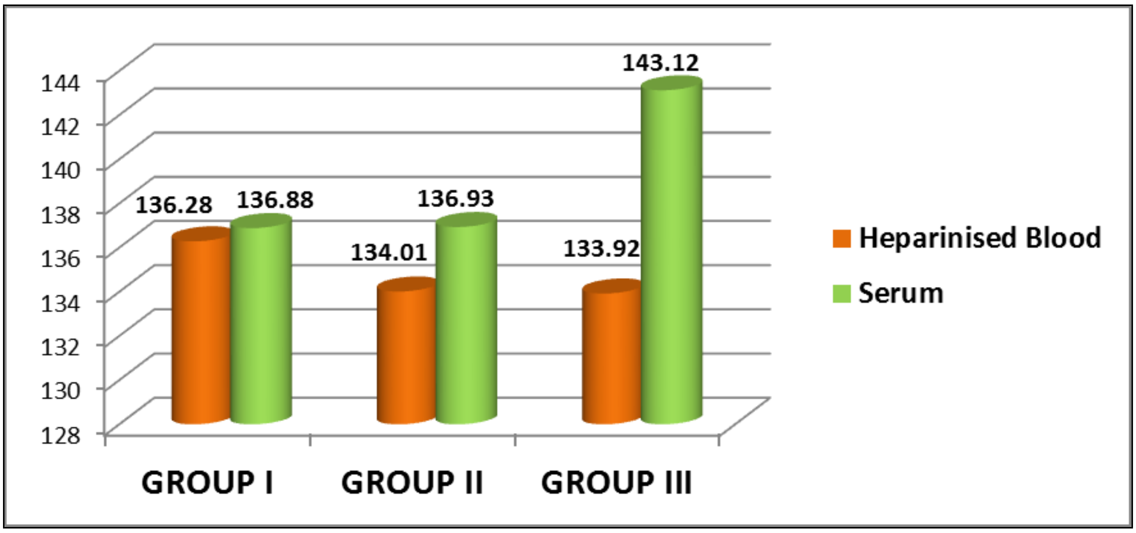

Figure No. 2: Graphical Comparison of Serum Sodium in heparinised blood and serum analyzed on electrolyte analyser in all the 3 groups.

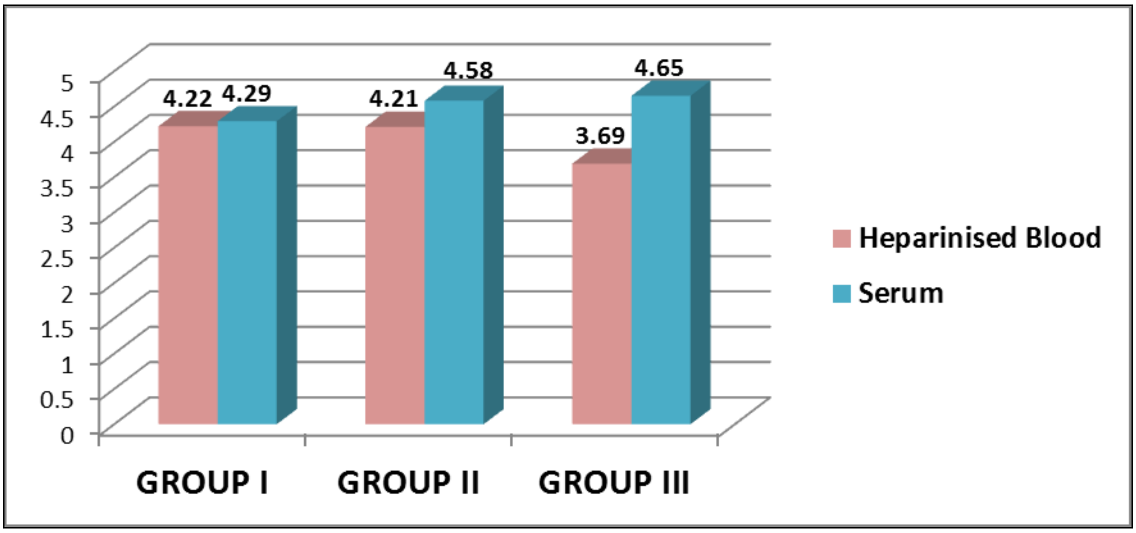

Figure No. 3: Graphical Comparison of Serum Potassium in heparinised blood and serum analyzed on electrolyte analyser in all the 3 groups.

In **Table no. 1**, The analysis showed that in group I (having a very low variation in electrolyte values between heparinised blood and serum) the mean of sodium measured on the electrolyte analyser with heparinised blood was 136.28 ±7.39 mmol/L,and the mean of sodium measured by the same electrolyte analyser with serum was 136.88 ±7.42 mmol/L. **(Figure no.2)** Their statistical analysis showed statistical insignificant differences with p value (>0.05).

As per **Table no. 2**, The analysis showed that in group II (having moderate variation in electrolyte values between heparinised blood and serum) the mean of sodium measured on the electrolyte analyser with heparinised blood was 134.01 ±7.72 mmol/L,and the mean of sodium measured by the same electrolyte analyser with serum was 136.93 ±7.62 mmol/L. **(Figure no.2)** Their statistical analysis showed statistical significant differences with p value (<0.05).

In **Table no. 3**, The analysis showed that in group III (having high variation in electrolyte values between heparinised blood and serum) the mean of sodium measured on the electrolyte analyser with heparinised blood was 133.92 ±7.21 mmol/L, and the mean of sodium measured by the same electrolyte analyser with serum was 143.12 ±7.39 mmol/L. **(Figure no.2)** Their statistical analysis showed statistical highly significant differences with p value (<0.001).

Again as per **Table no. 1**, The analysis showed that in group I (having a very low variation in electrolyte values between heparinised blood and serum) the mean of potassium measured on the electrolyte analyser with heparinised blood was 4.22 ±0.85 mmol/L, and the mean of potassium measured by the same electrolyte analyser with serum was 4.29±0.83 mmol/L. **(Figure no.3)** Their statistical analysis showed statistical insignificant differences with p value (>0.05).

In **Table no. 2**, The analysis showed that in group II (having moderate variation in electrolyte values between heparinised blood and serum) the mean of potassium measured on the electrolyte analyser with heparinised blood was 4.21±0.91 mmol/L, and the mean of potassium measured by the same electrolyte analyser with serum was 4.58±0.85 mmol/L. **(Figure no.3)** Their statistical analysis showed statistical significant differences with p value (<0.05).

In **Table no. 3**, The analysis showed that in group III (having high variation in electrolyte values between heparinised blood and serum) the mean of potassium measured on the electrolyte analyser with heparinised blood was 3.69 ±0.93 mmol/L, and the mean of potassium measured by the same electrolyte analyser with serum was 4.65 ±0.90 mmol/L. **(Figure no.3)** Their statistical analysis showed statistical highly significant differences with p value (<0.001).

LIMITATION OF THE STUDY

The limitation of our study was that sample size should be more so that more significant results may be obtained and there were very few studies available related to this research.

DISCUSSION

Fifty years ago, some researchers first reported that collection of blood gas samples for measurement of Arterial Blood Gases (ABG) and Electrolytes into the syringe with liquid heparin caused dilution error.[8-10] The Research Gate Discussion on correlation between electrolytes in blood gases and serum electrolytes ensured that the serum electrolytes are more reliable, while some argue accuracy depends on the machines. The lab results were discussed to be more accurate, but they are very much delayed and to start immediate treatment, hence; one has to rely on Blood Gas Machine.[11]

Some researchers notified recommendations about standardized blood collection into syringes washed with liquid heparin.[12,13] Also, **International Federation of Clinical Chemistry (IFCC)** recommended for blood gas sampling to fill dead space of the syringe with heparin, to lubricate the inner wall of the syringe, to expel the excess anticoagulant and to collect the least 20 times the dead space volume of blood.[14] Dead space of syringes is defined as the volume of liquid retained in the hub and needle when the syringe is completely emptied.[15]

Hamilton *et al.* and Ordog *et al.* recommended standardized blood collection into syringes washed with liquid heparin, since the FHC (Final Heparin concentration) in syringes washed with liquid heparin is considered to be sufficient.[12,13] IFCC recommends that FHC should be equal to 4-6 IU/mL liquid heparin in plastic syringes.[14]

Our present study was designed with an aim to observe the effect of heparin on electrolytes concentration in blood and to compare electrolyte concentration (Sodium and Potassium) measured in heparinised blood sample as well as serum sample of the same patient on the same electrolyte analyser. We also focused to find out the insignificant differences ($p>0.05$) between heparinised blood electrolytes and serum electrolytes so that heparinised blood electrolytes can also be trusted in place of serum electrolytes. Thus, reducing the work load of laboratory and benefitting patient economically.

In the study, done by **Alanazi et al** found a positively significant correlation between Na+, K+ and Ca++ measured both in the ABG and serum sample. They therefore concluded that critical decisions can be made by trusting the Na^+, K+ and Ca++ values obtained from both the arterial blood gas analysis and the serum. Likewise, in our study we have observed that there is a positive significant correlation between the electrolytes measured in heparinised blood as well as serum.[16]

Nanda et al.,[17] also found that arterial sodium and arterial potassium can be used instead of venous sodium and venous potassium levels in the management of critically ill patients. We are in favor of their study as we have observed that the differences in electrolytes in heparinised (arterial) blood sample and serum (venous) were insignificant ($p>0.05$) in group I, therefore, electrolytes values in heparinised (arterial) blood can also be taken into consideration in order to save time and patient's life.

Shek and Swaminathan studied errors due to heparin in the estimation of plasma sodium and potassium concentrations .They found that sodium concentrations of the 5000 and 1000 U/ml heparin preparations were 155 and 184 mmol/l respectively when analysed by flame photometry and undetectable and 167 mmol/L when analysed in the ISE. Similarly, Potassium concentrations of the 5000 and 1000 U/ml heparin preparations were 4.1 and 0.81 mmol/L respectively by flame photometery and 1.6 mmol/L and undetectable by ISE. From this an opinion can be made that heparin concentration alters electrolytes concentration (Sodium and Potassium) in blood.[18] In our study we found that Group I showed insignificant ($p>0.05$) results as compared to group II and group III which showed significant ($p<0.05$) and highly significant ($p<0.001$) results respectively. These above stated significant differences may be due to improper dilution of blood sample with heparin.

Previous studies suggest that heparin dilution also affects Na+, K + and ionic Ca^{++} varying from − 12% to 12%. Various authors have previously shown that estimation of Na^+, K^+ and Ca^{++} may be low in a sample collected for and analyzed by the blood gas machine. This has previously been attributed to binding of cations from the sample by liquid heparin.[19,20,21] Likewise our study also suggests that if heparin is used in more quantity (i.e > 50-80 IU/ml vol. of blood) low and incorrect electrolyte values may be obtained.

CONCLUSION

Correct heparin concentration in heparinised blood samples is very important for preventing coagulation. Due to insufficient addition of heparin, anti coagulation process does not take place as a result blood clots may occur which may cause analyzer failure *via* obstruction of tubings and capillaries of electrode membranes. In our hospital, in order to avoid clotting of blood sample they add more heparin to the syringes due to which blood sample gets hyperdilluted. Also, this may alter the Electrolytes (Sodium and Potassium) concentration in blood.

From our observations, we therefore concluded that critical decisions can be made by trusting the Electrolyte (Sodium and Potassium) values obtained from the heparinised blood analysis because the differences between the heparinised blood electrolytes and the serum electrolytes were statistically insignificant ($p>0.05$) i.e in Group I where correct heparin concentration(i.e 50-80 IU/ml vol. of blood) was used. If blood is diluted with more heparin, electrolyte (Sodium and Potassium) values will be lowered which are unreliable and unacceptable.

ACKNOWLEDGEMENT

We wish to thank the technical staff of Emergency Biochemistry Department of PGIMS,Rohtak for their support and **CL Micromed Pvt Ltd** (Delhi, India) for maintaining the machines throughout the study.

CONFLICT OF INTEREST

There are no conflicts of interests.

REFERENCES

1. D. Kapoor, M. Srivastava, P. Singh, Point of care blood gases with electrolytes and lactates in adult emergencies. Int J CritIllnInj Sci., 2014; 4: 216-22.
2. Bonini P, Plebani M, Ceriotti F, Rubboli F. Errors in laboratory medicine. Clin Chem, 2002; 48: 691-8.
3. Lippi G, Salvagno GL, Brocco G, Guidi GC. Preanalytical variability in laboratory testing: influence of the blood drawing technique. Clin Chem Lab Med, 2005; 43: 319-25.
4. Giavarina D, Villani A, Caputo M. Quality in point of care testing. Biochem Med, 2010; 20: 200-6.
5. H. Kamali, M. Mohri, Comparison of Plasma and Serum Biochemistry in Cat, Revue Méd. Vét., 2015; 166: 9-10, 275-279.
6. Toffaletti J, Ernst P, Hunt P, Abrams B. Dry electrolyte-balanced heparinized syringes evaluated for determining ionized calcium and other electrolytes in whole blood. Clin Chem, 1991; 37(10): 1730-33.
7. Scott MG, LeGrys VA, Klutts JS: Electrolytes and blood gases. In Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th edition. Edited by Burtis DE, Ashwood ER, Bruns DE. St Louis, MO: Elsevier, 2006; 983–1018.
8. Andersen OS. Sampling and storing of blood for determination of acid-base status. Scand J Clin Lab Invest, 1961; 13: 196-204.
9. Bradley JG. Errors in the measurement of blood P CO2 due to dilution of the sample with heparin solution. Br J Anaesth, 1972; 44: 231-2.
10. Hansen JE, Simmons DH. A systematic error in the determination of blood PCO2. Am Rev Respir Dis, 1977; 115: 1061-3.
11. I. Suresh, M.A. Tagin, J.Anabrees, Is there any correlation between electrolytes in the blood gases report and serum electrolytes report? Research Gate Discussion, June 2013.
12. Hamilton RD, Crockett RJ, Alpers JH. Arterial blood gas analysis: potential errors due to the addition of heparin. Anaesth Intensive Care, 1978; 6: 251-5.
13. Ordog GJ, Wasserberger J, Balasubramaniam S. Effect of heparin on arterial blood gases. Ann Emerg Med, 1985; 14: 233-8.
14. Burnett RW, Covington AK, Fogh-Andersen N, Kulpmann WR, Maas AH, Muller-Plathe O, et al. International Federation of Clinical Chemistry (IFCC). Scientifi c Division. Committee on pH, Blood Gases and Electrolytes. Approved IFCC recommendations on whole blood sampling, transport and storage for simultaneous determination of pH, blood gases and electrolytes. Eur J Clin Chem Clin Biochem, 1995; 33: 247-53.
15. Hall GH, Thompson CJ, Palmer A. Danger of dead space in U100 insulin syringes. Br Med J (Clin Res Ed), 1984; 288: 284-5.
16. Abdullah Alanazi1, Nowaf Yousef Al Obaidi2, Farhan Al. Enezi3, Mohammed Khalaf AlMutairi1, Nawfal Aljerian4, Omar Abdullah Alsultan5, Khalid alharbi6, Shoeb Qureshi7,* Correlation between the Measurements of Serum and Arterial Blood Gas (ABG) Electrolytes in Patients Admitted to the Intensive Care Unit at King Abdul-Aziz Medical City, Riyadh, Saudi Arabia, American Journal of Clinical Medicine Research, 2015; 3(3): 55-59.
17. S.K. Nanda, L. Ray, A.Dinakaran, Agreement of arterial sodium and arterial potassium levels with venous sodium and venous potassium in patients admitted to intensive care unit. J ClinDiagn Res., 2015; 9: BC28-30.
18. Shek C.C, Swaminathan R, Errors due to heparin in the estimation of plasma sodium and potassium concentrations. Intensive Care Med., 1985; 11: 309-311.
19. Hopper, K., Rezende, M. L., & Haskins, S. C. Assessment of the effect of dilution of blood samples with sodium heparin on blood gas, electrolyte, and lactate measurements in dogs. American journal of veterinary research, 2005; 66(4): 656-660.
20. L Andt, M., Hortin, G. L., Smith, C. H., McClellan, A., & Scott, M. G. Interference in ionized calcium measurements by heparin salts. Clinical chemistry, 1994; 40(4): 565-570.
21. Heining, M. P., & Jordan, W. S. Heparinization of samples for plasma ionized calcium measurement. Critical care medicine, 1988; 16(1): 67-68.