The Effects Of Speed And Duration Of Centrifugation On The Values of Some Commonly Measured Plasma Electrolytes

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Abstract—Centrifugation is a routine process in a clinical chemistry laboratory where blood specimens collected into anticoagulant containers are centrifuged to separate the plasma from the blood cells. This is done at various speeds, times and temperature which most times affect the quality of samples mainly due to haemolysis. World Health Organisation guidelines advocate for a maximum duration of 15 minutes as the centrifugation duration for separation of plasma. Various laboratories in this region adopt various speeds and duration for separation of blood samples without minding any possible effect it may have on the value of certain common analytes. This study was therefore designed to study the effect of different centrifugation speeds and duration (within the scope of the WHO centrifugation time guidelines) on the value of commonly measured analytes like sodium, potassium, chloride and bicarbonate. A cross-sectional study where blood was taken from 30 apparently healthy undergraduate volunteers after dividing the group into A and B of 15 subjects each. From the A group, blood specimens from each subject was separated based on the different centrifugation speeds and from the B group, blood specimens from each subject was separated based on different centrifugation times. The different samples from each subject were analysed for sodium, potassium, chloride and bicarbonate and this was done for all subjects. The mean values of all the analytes were about the same for the 1000 Revolutions per Minutes (RPM), 2000 RPM, 3000 RPM and 4000 RPM and so were the mean values of all the samples labelled 3 minutes, 6 minutes, 9 minutes, 12 minutes and 15 minutes. The differences in the means of the subgroups for group A and group B were statistically not significant. From this study, it can be advocated that plasma can be separated from whole blood samples at a maximum speed of 4000 RPM for 3 minutes duration without affecting the accuracy of most analytes and the introduction of this speed and duration will improve the quality assurance of laboratories in this region.

Index Terms—Centrifugation, turnaround time, and centrifuge.

I. INTRODUCTION

Centrifugation is one of the routine procedures in a clinical chemistry laboratory. It is mainly carried out at the pre-analytical phase of the total testing process in laboratories. A centrifuge can be said to be a device for the separation of particles from a solution according to their

size, shape, density, viscosity of medium and rotor speed. There are different types of centrifuges, - the desk top centrifuge, high speed centrifuge and ultracentrifuges [1]. One commonly used for separating whole blood in the laboratory is the desk top type. It is used to separate plasma from blood cells and this has been noted to be done at different revolutions per minute (RPM) and for different centrifugation duration (times). Most laboratories seem to determine the speed and time of centrifugation they use in their laboratories in this region. Blood samples are separated at a speed between 2000 to 4000 RPM for a time between 2 minutes to 10 minutes by different laboratories in this region. This means the speed of centrifugation and the duration of centrifugation varies with different laboratories. The World Health Organisation (WHO) guidelines propose at most, a 15-minute centrifugation time [2]. This guideline is believed to be expert's opinions or recommendations from manufacturers of laboratory centrifuges [3, 4]. Studies have suggested that centrifugation can directly affect certain analytes and this may be due to haemolysis which causes a release of intracellular analytes into the blood [5].

It is expected that blood samples received in the laboratory be centrifuged without delay as the more the delay, the worse the quality of samples. Studies have proved that the best quality of sample is gotten when whole blood is separated at most three hours after blood sample collection [6]. Of recent, various laboratories have carried out various types of studies on centrifugation speed, centrifugation time and temperature and have found all to affect sample quality to various degrees [7]. Most laboratory centrifuges are designed to have a centrifugation speed from 500 RPM to 4000 RPM and a centrifugation time from 1 minute to 100 minutes. Some centrifuges with faulty automatic time regulators are still in use in most resource constrained regions like ours and so are left to spin at a selected speed for various centrifugation duration (5 minutes or more) since the duration is monitored manually.

All these procedural lapses are expected to have various degrees of effect on sample quality. This study was therefore designed to evaluate how the various durations and speeds of centrifugation used to separate plasma from blood affect the value of certain common analytes like sodium, potassium, chloride and bicarbonates.

II. METHODS

This was a cross sectional study where 30 apparently healthy undergraduate volunteers who signed our consent forms after the importance of the study and the procedures

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involved were explained to them were selected. The study group was made up of 15 males and 15 females this was further divided into A and B subgroups.

Group A had 8 males and 7 females while group B was made up of 8 females and 7 males. From each subject in group 'A', 8ml of blood was drawn, 2ml each into 4 different vacutainer heparin containers and labelled tubes 1, 2, 3 and 4 for the 1000, 2000, 3000 and 4000 RPM respectively. Each tube was centrifuged for 3 minutes at different speeds of 1000, 2000, 3000 and 4000 RPM accordingly. The plasma in each tube was then transferred into plain tubes and again labelled accordingly. Sample in each tube was later same day analysed for sodium, potassium, chloride and bicarbonates. This was done for all the 15 subjects in that group.

For group B, 10ml of blood was collected from each subject, 2ml each was emptied into 5 different vacutainer heparin containers and labelled 3, 6, 9, 12 and 15 minutes according to the intending centrifugation time. Each tube was centrifuged at 4000 (Revolution Per Minutes) RPM for the duration of time on tube and plasma separated into a plain bottle and labelled according to the duration of time of separation. Samples in each tube was again analysed for sodium, potassium, chloride and bicarbonate and this was done for all fifteen subjects in that group. All subjects in Group A's results of the various analytes were grouped according to the speed of centrifugation, that is, 1000, 2000, 3000 and 4000 Revolution Per Minutes (RPM) groups.

For each subjects in group B, results of sodium, potassium, chloride and bicarbonate from samples 3, 6, 9, 12 and 15 were grouped accordingly and the each analytes mean calculated and compared with means of these analytes from other groups. This was done for all the subjects and the mean values of all the analytes in the various sub-groups in groups A and B were evaluated and compared.

III. RESULTS

TABLE A: MEAN RESULTS OF DIFFERENT RPMs BUT SAME TIME

Analyte	1000	2000	3000	4000	ANOVA
K	4.03±0.37	3.95±0.39	4.40±0.81	4.43±0.86	0.7366**
Na	137.7±5.41	137.7±4.8	138.5±4.8	138.2±5.2	0.9955**
Cl	102.7±2.8	102.5±2.6	103.1±1.9	102.8±2.8	0.9928**
HCO ₃	25.4±1.2	24.7±1.6	25.3±1.5	26.3±0.4	0.5727**

RPM- Revolutions per minute.

**Difference in the mean was not statistically significant (p- value> 0.05)

TABLE B: MEAN RESULTS OF DIFFERENT TIMES WITH SAME RPM-3000

Analyte	3mins	6mins	9mins	12mins	15mins-43000	ANOVA
Na	140.65±4.9	140.75±4.8	140.8±.6	141.025±4.3	141±4.6	0.9999**
HCO ₃	27.9±0.14	26.87±0.24	29.35±0.25	28.55±0.32	28.75±0.18	0.9963**
Cl	102.1±0.82	102.15±0.75	102.2±.21	102.475±0.01	101.97±0.90	0.9600**
K	3.605±2.4	3.62±2.4	3.57±2.42	3.62±1.83	3.64±1.48	0.5625**

**Differences of the mean values not statistically significant (p- value >0.05)

Group A subjects, were randomly sub-divided according to the centrifugation speed and group 'B' subjects were subdivided based on the duration of centrifugation. Analysis of variance (ANOVA) was used to compare the means of the different subgroups using the Epi Info vision 7 software

and a p-value of less than 0.05 was considered significant.

For group A, the mean concentration of potassium for all samples separated at 1000, 2000, 3000 and 4000 RPM were 4.03, 3.95, 4.40 and 4.43 respectively and the differences between these means values were not statistically significant (P-value> 0.05)(Table A). The mean bicarbonate concentration for 1000, 2000, 3000 and 4000 RPM were 25.4, 24.7, 25.3 and 26.3, in the same order (Table A). The mean sodium concentration for 1000 RPM was 137.7mmol/l and that for 2000 RPM, 3000 RPM and 4000 RPM are 137.7mmol/l, 138.5mmol/l and 138.2mmol/l respectively. The mean chloride concentration were 102.7mmol/l, 102.5mmol/l, 103.1mmol/l and 102.8mmol/l for the 1000, 2000, 3000 and 4000 RPM respectively. The analysis of variance (ANOVA) for the means of the various analytes in the various sub-groups were all found not to be statistically significant (P- value > 0.05) (Table A).

For group B, samples were separated using the same speed (4000 RPM) but different durations (times) at 3, 6, 9, 12 and 15 minutes. The mean sodium concentration for the 3, 6, 9, 12 and 15 minutes samples were 140.6mmol/l, 140.8mmol/l, 140.8mmol/l, 141.0mmol/l and 141.5mmol/l, respectively, while the mean bicarbonate concentrations were 27.9mmol/l, 26.9mmol/l, 29.4mmol/l, 28.6mmol/l and 28.8mmol/l for the 3, 6, 9, 12 and 15 minutes samples accordingly. The mean chloride and potassium concentrations were 102.1mmol/l and 3.6mmol/l for the 3minutes sample and 102.1mmol/l and 3.6mmol/l for the 6minutes sample (Table B). The 9, 12 and 15 minutes samples had a mean potassium concentration of 3.57mmol/l, 3.62mmol/l and 3.64mmol/l accordingly and a mean chloride concentration of 102.2mmol/l, 102.5mmol/l and 101.97mmol/l respectively.

On the analysis of variance (ANOVA), the differences in the means of the 3, 6, 9, 12 and 15 minutes sample for potassium, sodium, chloride and bicarbonate result were not statistically significant (P-value>0.05)(Table B).

IV. DISCUSSION

Previous studies [5, 7] have shown that centrifugation can affect the quality of samples and to this effect, this study was designed to evaluate how the different speeds of centrifugation and different durations of centrifugation affect the values of some common analytes routinely evaluated in our laboratory.

The A group had samples that were separated based on their speed, all centrifuged for 3 minutes at different speed, that is 1000, 2000, 3000 and 4000 RPM. Common analytes like potassium, sodium, chloride and bicarbonate in each sample type were measured and the mean value of each analyte for all samples were calculated. For potassium the mean values were about the same for all the different speeds and using the analysis of variance it was noticed that the differences in the means of samples based on the different speeds were not statistically significant. This finding was same for sodium, chloride and bicarbonate which were all analysed after samples were separated at various centrifugation speeds of 1000, 2000, 3000 and 4000 RPM for each patient. The mean results of each analytes in all sample types were about the same. (Table A) This is to say

that a centrifugation speeds of 1000 to 4000 RPM for 3 minutes had little effect on the quality of samples.

For B group the differences in the results based on time/duration of centrifugation was found not to affect the concentration of these analytes as the differences in their mean values were not statistically significant. The mean results for all the measured analytes in the 3 minutes samples (Sodium, Potassium, Chloride and Bicarbonate) were calculated and found to be about the same with the means of sodium, potassium, chloride and bicarbonate analysed in samples under the 6, 9, 12 and 15 mins) (Table B). This also infers that samples from the same individual can be separated at a centrifugation speed of 4000 RPM for different centrifugation time of 3, 6, 9, 12 and 15 minutes without significantly affecting the outcome. That is with centrifugation time within the scope of this study there is no significant effect on the accuracy of these analytes (sodium, potassium, chloride and bicarbonate) as the values were about the same for the different duration.

The speed and time adopted for separating blood samples by centrifugation in this region, are done without any study cited as a scientific proof for this practice in this area. This has made this study very important as it certifies the process of centrifugation in this area and reduces unnecessary wasting of time by centrifuging for longer time/duration when same sample can be separated for a shorter duration. This will improve laboratory services by reducing the turnaround time, importantly every laboratory process is aimed at achieving quality results at no distant time [8].

Most of the processes at the Preamalytical phase includes sample requesting, patient preparations, specimen collection, and transportation to the laboratory. Others are proper documentation and centrifugation, aliquating before analysis. Each of these processes are prone to errors but more importantly could be time consuming. Centrifugation alone in very busy laboratories can be very time consuming. Most laboratory centrifuges are designed to have speeds between 1000 RPM to 4000 RPM and each selected speed can be adjusted to run at a time ranging from 1 to 100 minutes [9]. Therefore the speed and minimum time at which samples can be separated with little or no effect on quality is very important in other to further reduce laboratory turnaround time [8, 10].

Though studies have showed that centrifugation can cause haemolysis [5], this fact was not noticed in this study as the speed of 1000 RPM to 4000 RPM and a time of 3, 6, 9, 12 and 15 minutes were found not to affect the quality of blood samples after separation as shown in the mean results of intracellular potassium for the different speed and duration. A speed and time beyond 4000 RPM and 15 minutes may affect the quality but this was not evaluated in this study as the study was limited to the maximum time of 15 minutes given by world health organization (WHO) as the maximum centrifugation time for separating blood samples [5].

Ensuring that the right sample from the right patient is taken, rightly analysed and interpreted at the right time is also very important and right time means at the shortest possible laboratory turnaround time. To this effect, the 6, 9, 12 and a 15 minutes centrifugation time will be a waste of time as the results of analytes at these different durations were about the same with the 3 minutes time samples.

Since centrifugation is a routine procedure in every laboratory and may be time consuming for laboratories that receives large numbers of blood samples, reduction in the centrifugation time without affecting the quality of sample will be a very important measure for every laboratory since this will reduces the laboratory turnaround time and improve overall laboratory quality assurance.

V. CONCLUSION

Analytes like sodium, potassium, bicarbonate and chloride were not affected by the centrifugation speed of 1000, 2000, 3000 and 4000 RPM at 3 minutes centrifugation time or at a speed of 4000 RPM within a centrifugation time of 3 minutes to 15 minutes. It can be said that, the quality plasma is not affected by the different speeds and centrifugation durations within the scope of this study. Based on this study, the introduction of a centrifugation speed of 3000 RPM to 4000 RPM at a duration of 3 minutes for separation of whole blood in our laboratories will not only guarantee quality plasma but will also reduce laboratory turnaround time since centrifugation is more of a routine process in clinical chemistry laboratories and have been shown to affect the turnaround time.

CONFLICT OF INTEREST

All authors hereby declare that this study has no conflict of interest.

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