

ORIGINAL RESEARCH

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The effects of different centrifugation temperatures on blood coagulation parameters

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Abstract

In this study we aimed to detect the effect of different centrifugation temperatures on coagulation parameters in the emergency laboratory. Duplicate samples were collected into sodium citrate tubes from 45 patients and centrifuged concurrently with non-cooling (group 1) and cooling centrifuges (Group 2) in Ankara Numune Training and Research Hospital emergency laboratory. Inner temperatures of the centrifuges were recorded during the study. Afterwards, PT, aPTT, D-dimer and fibrinogen values were determined. No statistically significant difference was detected between groups for any of the parameters tested (PT, aPTT, D-dimer and fibrinogen) ($p > 0,05$). Results of the present study show that there is no significant effect of internal temperature of the centrifuge device used on blood coagulation parameters.

Keywords: Coagulation, centrifugation, temperature, pre-analytical phase, sample handling

Introduction

Bleeding or thrombosis occurs in people with coagulation disorders. Coagulation factors and activation of platelets result in hemostasis [1]. Prothrombin time, active partial thromboplastin time, D-dimer and fibrinogen tests are used routinely to evaluate the coagulation panels.

It is important to give accurate and reliable results in the laboratory. The laboratory testing is actually partitioned into three phases which are (pre)pre-analytical, analytical, and (post)post-analytical phases [2]. The type of anticoagulant used, sampling technique including fasting state, length of the venous stasis, order of draw, sampling from a catheter, centrifugation, transportation, and storage time and temperature time and temperature prior to analysis are the main preanalytical variables which can affect the results of coagulation tests [3-5]. Centrifugation of the samples is an important part of the preanalytical phase and common guidelines recommend the centrifugation of samples at room temperature [6,7].

In the present study we aimed to detect the effect of different centrifugation temperatures on coagulation parameters in the emergency laboratory with the use of cooling and non-cooling centrifuge devices.

Material and Methods

The study was performed in Ankara Numune Training and Research Hospital Emergency Biochemistry Laboratory. Duplicated blood samples from 45 patients were drawn into citrated tubes by the same phlebotomist (Becton, Dickinson and Company, Franklin Lakes, NJ) using 21 gauge needles for coagulation tests. All of the fasting blood samples were collected in the morning of the same day. The samples were centrifuged in the non-cooled (group 1) (NF 1200; Nüve, Turkey) and cooled (group 2) (NF 1200R; Nüve, Turkey) centrifuges at 1500 x g for 15 minutes at the same time. After centrifugation, plasma was immediately analyzed. All test parameters were studied in duplicates in the same run of the analyzer. Group 1 was centrifuged at room temperature as suggested [6] where as Group 2 was centrifuged with a cooled centrifuge. The inner temperatures of the centrifuges were recorded during the study (Group 1: 22,7-47,0°C) (Group 2: 4,8-9,9 °C). Hemolyzed, lipemic, clotted and icteric samples were excluded from the study. The duplicated samples were analyzed in a synchronized manner with ACL TOP 700 (Werfen, Warrington, UK) analyzer using

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original reagents. PT, aPTT, D-Dimer and fibrinogen tests were studied. PT and aPTT were measured with photometric method, fibrinogen levels were measured with Clauss method and D-dimer levels were measured with latex based immunassay.

The study was approved by the local Ethics Committee of Ankara Numune Training and Research Hospital. The research protocol complies with the 2000 Declaration of Helsinki and written informed consent was obtained from all participants.

Statistical Analysis

The data were analyzed by using Statistical Package for Social Sciences (SPSS 18)(SPSS Inc., Chicago, IL, USA). The results are reported as median (min-max). Differences between groups were compared using Wilcoxon Signed Ranktest. P value less than 0.05 was considered statistically significant. Percent changes were calculated for each parameter with (Group2- Group1/ Group 1) *100 formula and the results were evaluated with acceptable

performance (AP) limits according to CLIA guidelines [8,9].

Results

The results of the study are shown in Table 1. The internal temperature of the centrifuge devices were recorded during the study. The temperature of non-cooled centrifuge (group 1) was recorded as 22,7-26 °C while the temperature of cooled centrifuge (group 2) was recorded as 4,8-7,7 °C. When the centrifugation temperature was recorded in concurrent runs, it was observed that the maximum temperature reached by the non-cooled centrifuge was 47,0 °C and that of the cooled centrifuge was 9,9 °C. There were no statistically significant differences for PT, aPTT, D-Dimer and fibrinogen results. The % differences were calculated and found to be within the desirable limits for bias derived from biological variation, with respect to current analytical quality specifications (Table 1) [9].

Table 1. Between group comparisons of the analytes

Analyte	Group 1	Group 2	P	Difference, %	Desirable Bias, %
PT (sec)	11.7(10.0-33.0)	11.9(10.2-34.7)	0.174	1.7	2.0
aPTT (sec)	31.5(24.6-65.0)	31.1(25.1-53.4)	0.674	1.3	2.3
D-Dimer (ng/mL)	100(30- 591)	100(13-606)	0.940	0	8.82
Fibrinogen(mg/dL)	313(139- 511)	317(151-511)	0.135	1.3	4.8

Values are expressed as median (min-max), Group 1: non-cooled centrifuge, Group2: cooled centrifuge PT:Prothrombin time aPTT:activated partial thromboplastin time, p<0,05 statistically significant

Discussion

Pre-analytical phase describes all the procedures before the initiation of the laboratory analysis and is responsible for the majority of laboratory errors [10-11]. Pre-analytical errors may occur throughout the testing process. For example, during test selection, patient preparation, sample collection, sample transport, centrifugation, and storage [12]. Centrifuges can have different rotor types like swing out and angle rotors. There are also centrifuge types such as cooled and non-cooled ones. Clinical & Laboratory Standards Institute (CLSI) guidelines recommend the plasma preparation by once-centrifugation of a whole blood sample in non-cooled centrifuges for processing routine coagulation assay [7].

Previously, Lippi et al. have evaluated the effect of different centrifugation temperatures (+4 °C, +12 °C, +25 °C) on routine coagulation test parameters and found that there was no significant effect of centrifugation temperature on PT, aPTT, D-dimer and fibrinogen parameters. Our findings are also similar to this study [6]. On the other hand, the study of Yılmaz et al. revealed that centrifugation temperature may affect some test results as ALT, TSH and FT4 [13]. Analyses at Lower temperatures are not recommended for most plasma-based coagulation assays because of possible cold activation of Factor VII, loss of von Willebrand factor, and platelet disruption [7,14]. In previous studie, PT was shown to be shortened on specimens carried on ice and this was devoted to activaiton of factor VII at cold [15]. On contrary to this, cooled centrifugation yielded prolonged PT results, although not

significant clinically.

Conclusion

In conclusion, pre-analytical factors in coagulation testing are critical as they may lead to diagnostic errors. Although results of the present study did not yield any significant differences in tested parameters at different centrifugation temperatures, it can be accepted as a pioneer one and future studies should be planned with enlarged sample size with various centrifugation temperatures among with the inclusion of other coagulation parameters such as coagulation factors.

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Competing interests

The authors declare that they have no competing interest

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Ethical approval

This study was conducted at Ankara Numune Teaching and Research Hospital with approval of the local Ethics Committee and suitable with the Declaration of Helsinki. (E-17-1293)

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