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Change of routine coagulation parameters in plasma samples with different hematocrit values

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ABSTRACT

Aim: There are many preanalytical variables affecting routine coagulation tests. Increased hematocrit (Htc) levels are one of these variables. However, no study has been conducted to determine the effect of low Htc values on coagulation tests. Therefore, in this study, we aimed to evaluate whether low Htc values affect coagulation tests besides high Htc values.

Methods: Standard human plasma was injected into coagulation tubes containing 3.2% sodium citrate to reflect hematocrit rates of 5% to 75% and prothrombin time (PT), active partial thromboplastin time (aPTT), thrombin time (TT) and fibrinogen measurements were performed.

Results: Three groups were formed according to Htc levels: A (5-25%), B (30-50%) and C (55-75%). PT (s) were found 13.7±0.30 in group A, 14.88±0.57 s in group B, 20.16 ±4.66 s in group C respectively. aPTT (s) results were 35.79±1.39 s in group A, 42.48 ± 3.51 s in group B and 76.47 ± 31.55 in group C. TT (s) results were found to be 26.42 ± 0.77 s (group A), 28.24±1.17 s (group B) and 32.02±2.60 (group C). Fibrinogen levels (g/L) were measured as 2.30 ± 0,05, 2.21 ± 0,07 and 1.90 ± 0.20 in groups A, B, C, respectively. For all measured parameters, group A reflecting low Htc was significantly ($P < 0,0001$) different from the other groups.

Conclusion: Previous studies have reported that high Htc (> 55%) levels affected routine coagulation tests. In our study, low Htc (5-25%) values were also shown to cause errors in the test results.

Keywords: preanalytical error, coagulation tests, low hematocrit levels, high hematocrit levels

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INTRODUCTION

Although systematic evidence of the specific contribution of laboratory medicine to clinical outcomes is difficult to obtain [1], clinical laboratory results affecting critical decisions such as patient admission, discharge and drug therapy, contribute significantly to the successful outcome of clinical medicine by constituting a determinant in finding a diagnosis, identifying a course of action for drug therapy, and monitoring the patient's response to treatment [2,3].

Quality is an issue of utmost importance for all clinic laboratories. The application of quality control to laboratory processes includes preanalytical, analytical and post-analytical steps. The preanalytical step, which starts with the thoughts of the doctor as to the selection of the test that will be demanded from the patient and which includes the sampling, transport and all procedures before the analysis, is an important component of the notion of total quality control. Studies have shown that most of the laboratory errors occur in the pre-analytical and post-analytic stages (46-68.2% and 18.5-47%, respectively), and fewer in the analytical stage (7-13.3%) [4].

Coagulation tests are performed in the presence of unexplained bleeding, in order to explain the abnormal test results detected during pre-operative and routine screening tests or for the follow-up of anticoagulant treatment.

Prothrombin time, activated partial thromboplastin time, thrombin time and fibrinogen levels are the coagulation tests with the highest use in the clinic. These tests are routine coagulation tests and if there is any abnormality in them, it is necessary to determine the reason for such abnormality by passing to more specific tests. It is difficult for clinicians to diagnose or provide effective treatment if the test results in the coagulation panel are not correct and reliable.

In modern coagulation laboratories, advanced devices and highly sensitive reagents are used and many problems in the analytical step have been resolved. However, unfortunately, these laboratories are still the clinical laboratories where most pre-analytical errors occur.

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In addition to sampling from the wrong patient or using inappropriate anticoagulant tubes for sample collection, incorrect sample volume, clotted samples, hemolysis, and lipemia, the patient's age, gender, ethnic group, blood group, physical activity level, stress status, circadian and diurnal rhythm and anticoagulant use are also suggested as preanalytical causes affecting coagulation tests [5,6]. Moreover, the hematocrit values of the patient are another pre-analytical factor that might influence test results [5,7].

Inaccurate results due to pre-analytical variables in coagulation laboratories may cause unnecessary further investigations, improper drug treatments, postponement of surgical interventions, and anxiety in patients. The severity of the erroneous results depends on the type of test that was performed, the magnitude of the difference between the actual result and the reported result, and whether the laboratory staff or clinician noticed the situation [5].

If the hematocrit values, which are one of the pre-analytical variables, are high, the anticoagulant ratio (proper blood / anticoagulant ratio = 9/1) is high due to the lower plasma volume in the sample, resulting in falsely prolonged clotting times. The prolongation of clotting time is due to the plasma/citrate volume ratio. In polyglobulic patients, there is less plasma for the standardized volume of citrate in the tube.

Clinical and Laboratory Standards Institute (CLSI) recommends adjusting the amount of citrate according to hematocrit in samples with hematocrit> 55%, in order to increase the sensitivity and accuracy level in hemostasis tests [8,9]. However, one in every four people in the world is diagnosed with anemia [10]. It is common for patients with low hemoglobin levels, to have low red blood cell counts; also, low hematocrit values are a more frequent situation than high Htc values. Although there are studies on setting the citrate level at high Htc values, it has not been evaluated whether there is a problem in the evaluation of the coagulation test results in patients with anemia. Therefore, in this study, the effect of change in plasma/citrate ratio in samples reflecting 5-75% hematocrit values that cover all low, normal and high hematocrit levels, on routine coagulation tests prothrombin (PT), activated partial thromboplastin (aPTT), thrombin time (TT) times, and fibrinogen will be examined.

MATERIAL AND METHOD

The entire experimental procedure was created using Standard Human Plasma (SHP) (Cat no: 363047, Siemens Healthineers Diagnostics Products GmbH, Erlangen, Germany). Obtained from the pooled citrated plasma which was collected from chosen healthy blood donors, SHP was stabilized with HEPES buffer solution (12 g/L) and lyophilized. Just before the study, the required amount of SHP was reconstituted by adding 1 mL of distilled water into each of them as recommended by the manufacturer. All dissolved SHPs were combined and mixed, and this mixture was used during the experiment. BD Vacutainer (Becton Dickinson, Franklin Lakes, NJ, USA) tubes with a total volume of 1.8 mL containing 3.2% citrate were used for the tests.

One unit of whole blood, taken from one of the study researchers (CN) and placed in a blood bank bag containing acid citrate dextrose was placed in 50 ml disposable falcon tubes, centrifuged at 4000g for 10 minutes, and the plasmas were discarded. Erythrocyte pellets were prepared by washing the remaining cellular elements in Falcon tubes twice with isotonic NaCl.

Packs of erythrocytes in falcon tubes were combined. The erythrocyte packages were placed in the citrated tubes in amounts to provide the calculated %Htc. HSP was added to complete the total volume of the tube to 1.8 ml (for example, for the tube containing 30% Htc, 1.26mL SHP was added on top of 0.54 mL erythrocyte package). The number of tubes prepared according to Htc levels were given in Table 1. Following rotation in the mixer for 10 min, the tubes were centrifuged to separate the plasmas. Coagulation tests were studied in these plasmas. Since the main purpose of this study was to evaluate the change at low (<25%) Htc levels, 75 tubes with low (<25%), 60 with 30-50% and 48 with >55% Htc values were prepared for the experiments (Table 1). Each parameter was studied 3 times from the prepared citrated plasmas.

Although it is not possible to have 5% Htc values in routine clinical practice, we have planned the present study as an experimental one to include Htc levels as wide as possible. Therefore, we increased Htc levels in tubes by 5% at each step, in order to include a Htc range from 5% to 75%.

Citrate tubes reflecting the hematocrit value 5-25% were included in group A (n=75); whereas those reflecting %30-50 were included in group B (n=60) and those reflecting %55-75 were included in group C (n=48).

Routine coagulation tests were performed with reagents Thromborel® S [for prothrombin time (PT)], Dade Actin FS [for activated partial thromboplastin time (aPTT)], BC Thrombin [for thrombin time (TT)] and Multifibren U [for fibrinogen], by standard procedures on the Siemens BCS XP Analyzer Automated Coagulation System (Siemens Healthineers Diagnostics Products GmbH, Erlangen, Germany). SI units were used in this study.

Intra-assay and inter-assay coefficients of variability (CVs) of PT, aPTT, TT and fibrinogen kits were 2.02%

Table 1. Number of sample tubes prepared according to hematocrit (Htc) levels.

Htc levels: <25%		**Htc levels: 30-50%**		**Htc levels: 55-75%**	
Htc levels	**n**	**Htc levels**	**n**	**Htc levels**	**n**
5%	15	30%	12	55%	10
10%	15	35%	12	60%	10
15%	15	40%	12	65%	10
20%	15	45%	12	70%	10
25%	15	50%	12	75%	8
Total	75	Total	60	Total	48

n= Number of sample tubes

(mean control value of PT: 13.5 s) and 2.7% (mean control value of PT: 12.5 s), 1.3% (mean control value of aPTT: 34 s) and 4.6% (mean control value of aPTT: 27 s), 2.6% (mean control value of TT: 25.8 s) and 6.9% (mean control value of TT: 21.6 s) and 1.65% (mean control value of fibrinogen: 2.30 g/L) and 5.9% (mean control value of fibrinogen: 2.48 g /L), respectively.

This was an in vitro experimental study using commercial human plasma samples requiring no ethical consent.

Statistical analysis

Data analysis was made by using GraphPad Prism 5 software program (Graph Pad Software, San Diego, CA). The data were presented as number, percentage and arithmetic mean ± standard deviation (SD). The Student's t-test and one-way ANOVA were used for the comparison of continuous variables between two and more groups, respectively. The significance level was defined as $P<0.05$.

RESULTS

PT (s) was found in group A (n = 75): 13.70 ± 0.30, in group B (n = 60): 14.88 ± 0.57, in group C (n = 48): 20.16 ± 4.66 (Figures 1a and 2a).

aPTT (s) was measured in group A: 35.79 ± 1.39, in group B: 42.48 ± 1.39, in group C: 76.47 ± 31.55 (Figures 1b and 2b).

TT (s) was found in group A: 26.42 ± 0.77, in group B: 28.24 ± 1.17, and in group C: 32.02 ± 2.60 (Figures 1c and 2c).

Fibrinogen levels (g/L) were measured as 2.30 ±0.05, 2.21 ± 0.07 and 1.90 ± 0.20 in groups A, B, and C, respectively (Figures 1d and 2d).

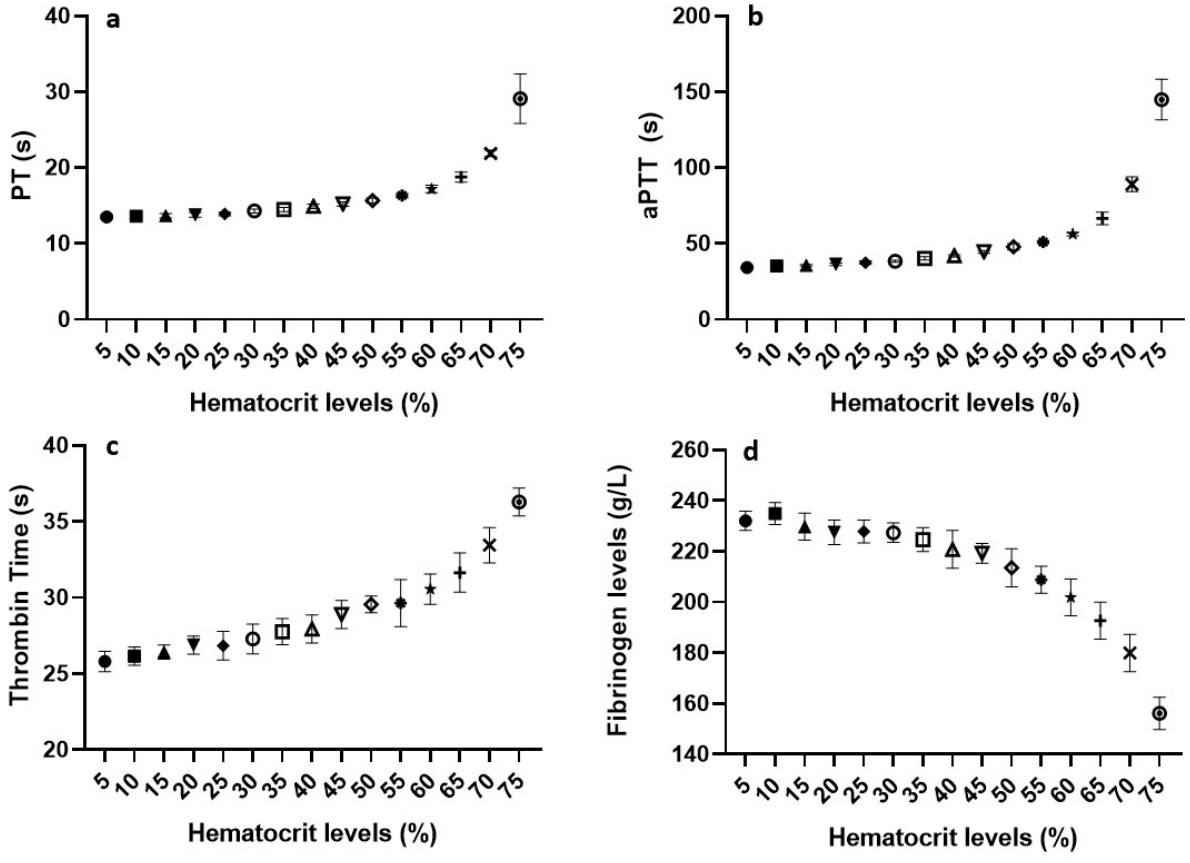

Fig. 1.(a) PT, (b) aPTT, (c) TT and (d) fibrinogen levels at different hematocrit values (mean ±SD).

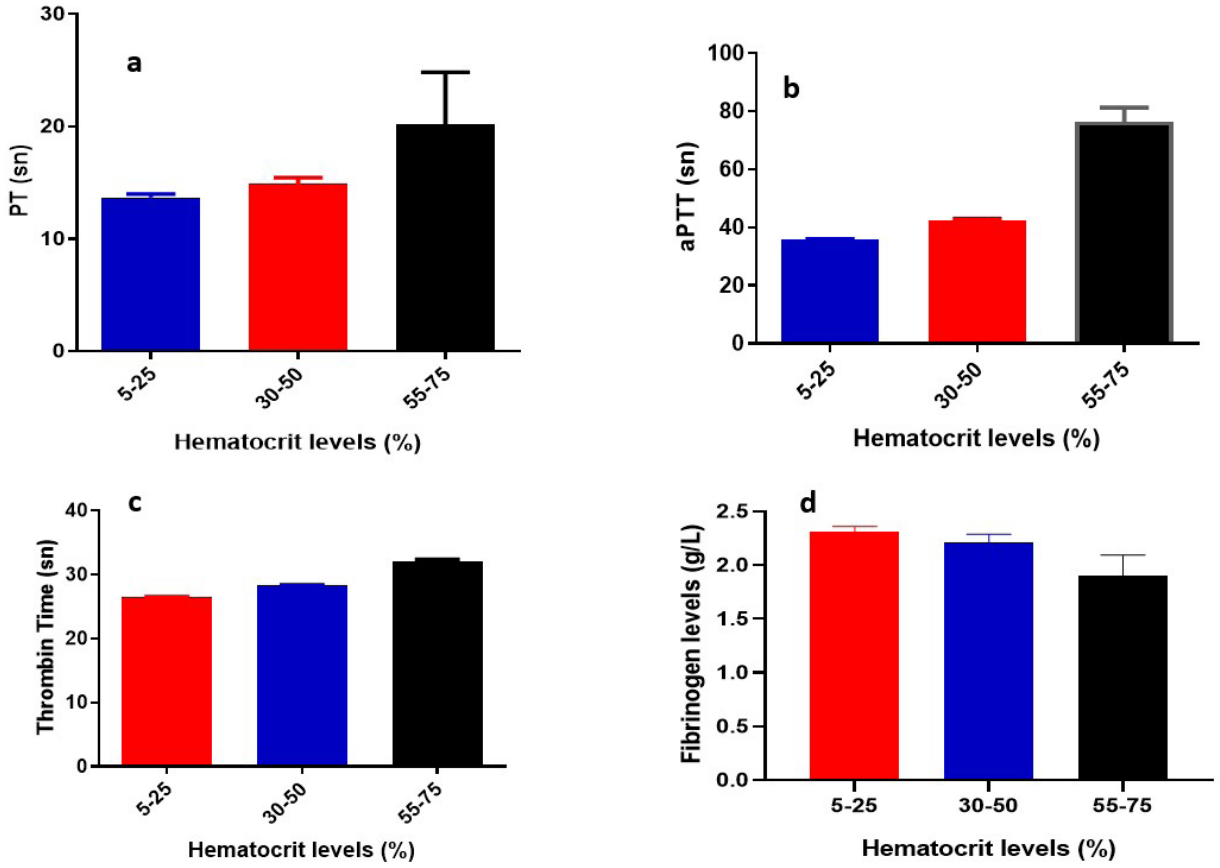

Fig. 2. (a) PT, (b) aPTT, (c) TT and (d) fibrinogen levels in A, B and C groups (mean ± SD)

For all measured parameters, significant differences (P <0.0001) were noticed between all groups reflecting low (group A), normal (group B) and high (group C) Htc. Compared to group B, it was determined that there was an 8% decrease in PT, 15.7% decrease in aPTT, 6.4% decrease in thrombin time and 4.1% increase in fibrinogen in group A. Compared to group B, there was a 35.5% increase in PT, 80% increase in aPTT, 13.4% increase in TT and 14.3% decrease in fibrinogen in group C. Compared to group A, it was determined that there was a 47.2% increase in PT, 113.7% increase in aPTT, 21.2% increase in TT and 17.7% decrease in fibrinogen in group C.

DISCUSSION

In modern coagulation laboratories, advanced devices, highly sensitive reagents and internal and external quality control applications are used in the analytical phase. Accordingly, the level of erroneous results for this stage has decreased significantly. However, as in other clinical laboratories, pre-analytical variables are still the most important reasons for erroneous patient results. Due to the use of tubes containing anticoagulants, the necessity of taking a certain amount of blood for each tube and the necessity to mix blood and anticoagulants properly, hemostasis laboratories are one of the laboratories where pre-analytical errors are most common. Blood collection for hemostasis tests under improper conditions causes significant problems. However, compared to other processes, preanalytical processes are relatively more difficult to standardize, since these require the participation of non-laboratory units.

When it comes to coagulation tests, sodium citrate (3.2%) constitutes the most commonly used anticoagulant. Standard evacuated tubes for coagulation assessments should contain 0.5 mL sodium citrate anticoagulant and 4.5 mL of blood in order to ensure that the blood/sodium citrate rate is 9 / 1 [8,11].

Furthermore, plasma obtained from tubes with anticoagulants such as EDTA and heparin is generally improper for most hemostasis tests [12].

In previous studies, it has been stated that the preanalytical error rate of coagulation tests is in the range of 2-5.5% [13]. About 5 to 13% of all improper samples received by clinical laboratories are due to the collection of samples into erroneous types of tubes. This accounts for 2% of all samples received in the coagulation laboratories [13,14].

Our laboratory is a large-scale university hospital laboratory where an average of 1600-2000 routine coagulation parameters are studied per day. An average of 2.31% of the samples that come to our laboratory for coagulation tests are rejected for being insufficient, clotted,

hemolyzed or lipemic. Approximately 18% of these consist of samples that are not taken in sufficient quantity.

Unlike incorrect drug treatment or mishandled surgery, laboratory errors do not always and necessarily cause severe problems for the patients. Thus, it is difficult to establish a direct connection between patient outcomes and erroneous test results. However, this does not mean that incorrect test results are insignificant, since these may lead to situations which may still be clinically meaningful and put laboratories at risk by causing various undesirable clinical results or adverse economic consequences [6,15].

Serious consequences may occur due to errors in routine coagulation testing as well. For instance, an incorrectly prolonged "screening" coagulation test may cause more cost and delay as well as unnecessary anxiety in the investigated patient as it may lead to a further clinical decision to undertake costly and time-consuming investigations such as specific diagnostic. Depending on the direction of the error, a patient being monitored for anticoagulant therapy may be put at risk of thrombosis or bleeding since an incorrect low or high coagulation test time may result in subsequent inappropriate dosing of anticoagulant therapy. A false-normal screening test result may prevent further analysis of factor assays, therefore erroneously discounting hemophilia and probably putting a patient at a needless risk of bleeding due to invasive medical processes such as surgery, biopsies, or dental extraction [6].

Sodium citrate (3.2%) is the most commonly utilized anticoagulant in clotting-based coagulation tests. The fill volume of sodium citrate as well as the ratio of sodium citrate:plasma constitute crucial pre-analytical points that are related to the process of blood sampling [16]. It has been mentioned that a change between the citrate-adjusted coagulation test result that is greater than 10% constitutes a clinically significant difference [16,17].

Therefore, all factors that will change the sodium citrate: plasma ratio may affect the results of coagulometric coagulation tests. As mentioned above, Htc levels are one of these factors and it is a parameter which should be considered in coagulation laboratories. In this study, it has been stated that not only high Htc values may affect the coagulation test results, but also low Htc values. As a consequence of the excess citrate relative to the residual plasma fluid in the sample, clotting times may prolong significantly in samples with an Htc value higher than 55%, which generally belong to neonates, burn patients and high-altitude residents or relevant to cases of serious dehydration and polycythemia vera [5].

Anemia is defined by the World Health Organization (WHO) as a hemoglobin (Hb) level of less than 12 g/dL in women and less than 13 g/dL in men [18]. As a common symptom affecting approximately %25 of the overall population [10], anemia was observed in 58.4% of hospitalized patients [19], and up to %72 of elderly hospitalized patients [20]. WHO classifies anemia as "mild" (9.5-10.9 g / dL), "moderate" (8.0-9.4 g / dL) and "severe" anemia (<7.9 g / dL) based on hemoglobin levels [21]. Hb <6.5 g / dL was added to this classification as a life-threatening type of anemia in the National Comprehensive Cancer Network (NCCN) Guidelines [22].

In our study, very low Htc levels were also evaluated in addition to normal and high Htc values. Although these very low levels are encountered in very few patients, life-threatening anemia may occur especially due to blood loss after acute severe trauma; hemolysis or hemorrhage in cancer patients and erythropoietin deficiency in patients with chronic renal failure. Coagulation tests may lead to erroneous results in all these patients.

In this study, it was determined that compared to the group where Htc levels are 30-50%, there was a significant change in aPTT levels in the group where Htc levels are 55-75%. PT results also changed remarkably although not as much as those of aPTT.

Similarly, the most significant change was found in aPTT levels when the group with low Htc levels (Htc 5-25%) and the group with normal Htc levels (Htc 30-50%) were compared. Even though the change was not as radical as in the group with high Htc values, the difference between the groups was also significant at lower Htc levels. Therefore, the evaluation of Htc levels appears as an important parameter for all patient samples to be studied with a coagulation test. As mentioned above, insufficient quantity is an important reason for the rejection of samples that come to our laboratory for coagulation tests. Moreover, while rejecting these samples, the Htc levels of the patients were not taken into consideration. Considering the Htc levels may result in more samples being rejected.

The ability of large laboratories like ours to check Htc levels in each sample is limited due to the fact that these are mostly evaluated in automatic devices where the samples are loaded into the racks these devices. However, Htc levels can significantly change coagulation test results in patients with both low and high levels. The finding that coagulation test results change in patients with low Htc levels as well as in those with high Htc levels will contribute to providing more accurate and reliable results in clinical laboratories by enabling the evaluation of Htc levels in coagulation devices. The Htc levels of 4.8% of the patient samples coming to our laboratory were in the range of 20-24.9%, and 0.7% of them were in the range of 10-19.9%. Htc levels were found to be <25%

in 5.5% of all patient samples. This surprisingly high rate of samples with less than 25% Htc levels suggested that low Htc results may affect coagulation tests in a significant number of patients.

Since they help evaluate whether a patient is at a risk to bleed or clot by assessing the performance of the hemostatic process, coagulation test results constitute a crucial determinant in the clinical decision-making process. Inaccurate results in these tests pose important risks for patients by causing erroneous clinical evaluations in oral anticoagulant therapy monitoring, screening and diagnosis of hemorrhagic and thrombotic disorders.

In conclusion, in accordance with the previous studies reporting an interaction of high Htc levels with routine clotting tests and adjustment of citrate concentration in samples with Htc> 55% to eliminate this error, we have observed a similar interaction of samples with low Htc (5-25%) levels with a resultant potential to cause errors in the test results, as well.

The finding of a significantly high ratio (5.5%) of blood samples with Htc levels less than 25% among all patient samples in our laboratory suggested that low Htc results may affect coagulation tests in a significant number of patients. Therefore, it may also be necessary for this group of samples to adjust the amount of citrate placed in the sample tubes and that the Htc levels of each sample coming to the laboratory for coagulation tests should be evaluated carefully.

AUTHORS' CONTRIBUTION

IOK- The conception and design of the work, the acquisition and analysis of data.

CN- Drafting the work, interpretation of data, revising it critically for important intelectual content.

SO- The conception of the work, revising, final aproval of the version to be published.

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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