Discrepancy in Optical & Mechanical Method in Coagulation Tests in a Turbid Sample

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Abstract Automated coagulation analyzers have replaced manual methods to meet the ever-increasing test load in many laboratories. Of the different methods, two distinct methods exist based on optical and mechanical clot detection (magnetic steel ball method). In optical method the detection of clot formation is measured by a change in optical density (OD) of a test sample. Mechanical clot detection technology, involves monitoring the movement of a steel ball within the test solution using a magnetic sensor. There are limited studies comparing both these methods and they have conflicting results regarding the effect of plasma turbidity on the final result. We report a case where a plasma factor (lipemia) caused prolongation of both PT and APTT, as measured by the photo optical method.

Keywords Lipemia · Automated coagulation analyzers · Photo optical method · Mechanical method

Introduction

Automated coagulation analyzers have replaced or are in the continual process of substituting the manual methods to meet the ever-increasing test load in many laboratories. Two of the most important tests that are frequently requested include prothrombin time (PT) and activated partial thromboplastin time (APTT). The modern analyzers

adopt different technologies to measure these two parameters. The advantages of automated coagulation analyzers include minimal manual interference which drastically reduces the chances of errors, an increased precision which ensures reproducibility, reduced personnel to do the task and importantly, a large number of samples that can be processed at the same time.

Of the different methods, two distinct methods exist based on optical and mechanical clot detection (magnetic steel ball method). [1] In photo optical method, detection of clot formation is measured by a change in optical density (OD) of a test sample. As the plasma sample clots, it becomes more optically dense & the amount of light falling on a photo-sensitive detector decreases (i.e. transmitted light decreases). The drop or change in light is determined as the endpoint of coagulation. The time taken for endpoint of coagulation is calculated in seconds. Thus, any plasma or cellular factor which increases the turbidity of sample can affect the test result.

Mechanical clot detection technology on the other hand, involves monitoring the movement of a steel ball within the test solution using a magnetic sensor. As clot formation occurs, the fibrin strands formed alters movement of the ball, which is detected by the sensor. The time taken for end-point of coagulation is calculated in seconds. By its fundamental principle, the chances of plasma turbidity influencing the final test result is comparatively low or nil.

There are limited studies which compare these two methods. Some studies [1–3] have suggested that optical and mechanical detection methods are equivalent in terms of correlation, accuracy, and precision for coagulation testing and that both are unaffected by sample turbidity.

We report a case where a plasma factor caused prolongation of both PT and APTT, as measured by the photo optical method.

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Fig. 1 Blood sample with turbid plasma due to lipemia

Case Report

A 37 year old male patient presented to medicine outpatient department of Kasturba hospital with complaints of decreased appetite, generalized tiredness and increase in drinking habits since 6 months. On examination, mild icterus was appreciated. Liver was palpable 2 cm below right costal margin. It was firm with straight border and smooth surface. Spleen was not palpable. A clinical diagnosis of alcoholic liver disease was made. Routine blood investigations were ordered including coagulation profile. For measurement of PT and APTT, as requested by the clinician, the venous blood was collected into a tube containing 3.2 % sodium citrate. PT and APTT were measured using optical mode of Amax Destiny PlusTM analyzer. Both PT and APTT were more than 120 s. As a laboratory policy, any PT and APTT prolongation is confirmed by using the mechanical mode, available on the same coagulation analyser. Thus, the sample was run on mechanical mode of Amax Destiny PlusTM analyzer. PT was 17 s (control: 15.9 s) and APTT was 34.4 s (control: 32.3 s).

This discrepancy pointed towards a possible plasma variable. On gross examination, the plasma was turbid (Fig. 1). The ensuing biochemical investigations revealed that the total cholesterol was more than 818 mg/dl and HDL cholesterol was 39 mg/dl. Total bilirubin was 1.5 mg/dl, direct bilirubin 1.4 mg/dl, ALT 12 IU/L, AST 10 IU/L, Serum Albumin 3.60 g/dl. Hence, lipemia was incriminated as the plasma factor which caused a discrepant result in both clot detection methodologies.

Discussion

Every laboratory is usually inundated with requests for PT and APTT. Automated coagulation analyzers provide distinct advantages and mode options especially in a high-volume coagulation testing centre. The mechanical and photo-optical modes are two distinct methodologies, available in a single analyzer such as Amax Destiny PlusTM (Trinity Biotech); currently used in our laboratory. In photo optical method the clot formation is measured by change in OD of the sample. In mechanical method the clot formation is measured by monitoring the movement of steel ball by magnetic sensor. Though both methodologies have co-existed since the 1960s, there is a continual debate regarding the superiority of optical method as compared to mechanical method and vice versa. There have been studies comparing both methods suggesting the superiority of mechanical method especially in case of turbid lipemic, icteric and haemolytic samples [4, 5]. Other studies demonstrated no significant interference due to lipemic or haemolytic sample on PT and APTT measurements by photo optical method [1, 2, 6]. Some studies uphold photo-optical method especially in dysfibrinogenemia and sepsis [7].

We found that invalid PT and APTT results were obtained with total cholesterol of more than 818 mg/dl. Turbid samples interfere with the absorption of light in photo optical method. This prevents the instrument from seeing a drop or change in light as the sample clots. This leads to falsely prolonged results. In a study conducted by Dorn Bineke, et al. it was shown that triglyceride levels >11.4 mmol/L resulted in invalid PT measurements with sysmex CA-7000 analyzer using photo optical method [5]. Giuseppe Lippi, et al. [8] studied the effect of hemolysis on coagulation parameters using a photo optical method of measurement. They found that although slightly haemolysed specimens might be analysable, a moderate blood cell lysis, as low as 0.9 %, influences the reliability of routine coagulation testing. Thus, the efficacy of mechanical method for testing end-point of coagulation proved useful in this particular case.

Conclusion

There are discrepancies in the measurement of PT and APTT using photo-optical and mechanical modes in coagulation analyzers with reference to a turbid sample. This emphasizes the importance of pre-analytical phase of blood collection which helps to minimize these discrepancies. Also, in cases with invalid results with one method, a repeat test with the other method is recommended.

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