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Correlation Between Photo-Optical and Mechanical Automation Methods in Coagulation Testing in a Turbid Sample

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Abstract

Objective: To determine the correlation between photo-optical and mechanical methods of automated coagulation system for prothrombin and activated partial thromboplastin time in turbid samples.
Methodology: This cross sectional study was conducted in department of hematology, Lahore General Hospital, Lahore from 20-10-2023 to 20-4-2024. One hundred patients fulfilling selection criteria were enrolled. Patients' samples were divided in two parts and assessed on LINEAR coagulation analyzer, photo-optical clot detection and second part on SysmexCA 50, viscosity-based (mechanical) clot detection. SPSS 21 was used to input and analyse the data. PT, and APTT were presented as mean ± standard deviation. The association between the mechanical and photo-optical coagulation testing techniques for both PT and APTT, Pearson's correlation coefficient was computed. A p-value of less than 0.05 was deemed significant.
Results: Age range varied from 38.83±10.29 years. Among 100 patients 52(52%) were male and 48(48%) were female. Very low positive correlation was found between Optical and mechanical methods for PT. i.e. (r=0.010, p-value=0.918) in turbid sample. However APTT showed significant positive correlation between Optical and mechanical methods. (r=0.530, p-value=0.000)
Conclusion: Results of this study demonstrate low correlation between photo-optical and mechanical methods for PT (r=0.010, p- value=0.918) and significant correlation for APTT (r=0.530, p- value=0.000).
Keywords: Photo-optical, Mechanical, Coagulation, Prothrombin, Activated partial thromboplastin, Turbid, Samples.

Introduction

Coagulation is a fascinating process in which blood undergoes a remarkable transformation, transitioning from a fluid state to a solid blood clot. Understanding the process of coagulation requires a deep knowledge of the intricate interplay between platelets and clotting factors in the blood. Coagulation starts immediately after an injury that harms the endothelium lining of a blood vessel.[1] Damage results in blood being exposed to the damaged subendothelial space, which sets off two processes: platelet structural alterations and subendothelial tissue factor exposure to plasma factor VII, which triggers a cascade of clotting events that eventually result in the creation of cross-linked fibrin. RBC and platelets are ensnared by this fibrin, forming a clot. [2,3]

Disease conditions known as disorders of coagulation may cause obstructive clotting (thrombosis) or bleeding (bruising or haemorrhage). Prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time, platelet count, platelet functional test by PFA-100, thrombodynamic test, and other tests are used to evaluate the coagulation system's performance. [4] The effectiveness of the (intrinsic) route started by activation of the plasma "contact factors" (factor XII, factor XI, prekallikrein, and high molecular weight kininogen) is measured by the activated partial thromboplastin time, or APTT. The (extrinsic) process that is started by tissue factor release is measured by prothrombin time (PT). With the use of automated analysers, these tests may be carried out both manually and automatically. [4]

The availability of new, dependable equipment and an increase in workload have led to a growth in automation in laboratory work. There are two different automated technical approaches based on clot detecting techniques: mechanical and optical. [5,6] A change in the optical density of a test sample is used in the photooptical approach to identify the development of clots. Conversely, mechanical clot detection technology uses a magnetic sensor to track the movement of a steel ball within the test fluid. The fibrin strands that develop when the clot forms change the ball's motion, which the sensor detects.[7]

Authorship Contribution: [1,2]Conceived and planned the idea of the study, [3]final approval of the version to be published, Collecting the data, drafting the work or revising it critically for important intellectual content, [4-6]Literature review,

Funding Source: none
Conflict of Interest: none
Received: Sept 17, 2024
Accepted: Dec 27, 2024

When turbid samples are examined, disparities are discovered despite the high reliability and reproducibility of the automated findings. Samples including proteins, lipemia, jaundice, and hemolysis are considered turbid. This highlights how crucial the pre-analytical stage of blood collection is in reducing these disparities. It's also advised to repeat the test using the alternative approach if the first method yields erroneous findings. [7] According to Bai et al., there was a strong correlation between photo-optical clot detection and electro-mechanical detection systems (R2< 0.96 for all tests, i.e. =0.98) Even while assessing turbid samples, the two clot detection systems' correlation was steady (R2<0.98 for all tests, or r=0.99). [8]Excellent correlation between the photo optical and mechanical analysers for PT (R2=0.97, or r =.98), and aPTT (R2=0.85, or r = 0.92) has been recorded. As a result, even when assessing turbid samples, the two clot-detection systems' correlation held (R2 > 0.97, or r=0.98 for two tests). [9]

Literature shows that there is very strong positive relationship between optical and mechanical methods for assessment of PT and APTT in turbid sample, but not much work has been done in this regard. Also, there is lack of evidence in local literature and we are unable to implement the reliable method. Old methods are still applied in routine for assessment of coagulation testing. There is a need to conduct a study to determine the relationship so that in future more cost effective and time effective method can be applied which is also readily available on all set-ups. Through this study we want to have regional research results and evidence for recommending and guiding the laboratory for better results.

Methodology

This cross sectional study was conducted in department of hematology, Lahore General Hospital, Lahore from 20-10-2023 to 20-4-2024. Non-probability, consecutive sampling technique was used. A sample size of 100 is determined using a 5% significance level for type I error, a 10% significance level for type II error, and using a correlation coefficient of 0.92 between the optical technique and mechanical method in coagulation testing for APTT in blood samples.

Inclusion criteria: Adult patients of aged 14-75years of either gender having analytical interference in samples send for coagulation were included. Coagulation testing is the time required for the development of a fibrin clot is determined by either an optical or mechanical approach, using PT and APTT measurements.

Exclusion criteria: Samples of patients having other coagulation disorders i.e hemophilia, VWD, DIC etc were not included in the study.

Total 100 patients fulfilling selection criteria were enrolled. Informed consent was obtained before taking blood sample. Blood samples were obtained by using 5cc disposable syringe under aseptic measures and stored in sterile vials. All samples were divided in two parts and assessed on LINEAR coagulation analyzer, photo-optical clot detection and second part on Sysmex CA 50, viscosity-based (mechanical) clot detection. Turbid sample is one which causes plasma to be unclear or hazy due to the presence of impurities. For example Heamolysis (rupture or destruction of red blood cells), lipemia (presence of abnormally high levels of emulsified fat in blood i.e. LDL >150mg/dl, Triglyceride >200mg/dl) and jaundice (high level of bilirubin in blood i.e. total bilirubin >5mIU).Reports were assessed and PT and APTT was noted. Prothrombin time (PT)assessed the time it takes for clotting to occur in recalcified plasma when tissue extract (thromboplastin) is present. It provides an indication of the overall effectiveness of the extrinsic system. Activated partial thromboplastin time (APTT) evaluated the time it takes for plasma to clot following the activation of contact factors and the addition of phospholipid and CaCl2. However, tissue thromboplastin is not included in this test. The results of this test demonstrate the effectiveness of the intrinsic route. All this information was recorded on proforma.

Data analysis: SPSS 21 was used to input and analyse the data. PT, and APTT were presented as mean ± standard deviation. The association between the mechanical and photo-optical coagulation testing techniques for both PT and APTT, Pearson's correlation coefficient was computed. A p-value of less than 0.05 was deemed significant.

Results

Mean age of patients was 38.83±10.29 years. Minimum and maximum age of patients was 14 and 78 years. Among 100 patients 52(52%) were male and 48(48%) were female. Out of 100 patients 33(33%) were smokers.

Very low positive correlation between Optical and mechanical methods for PT. i.e. (r=0.010, p-value=0.918) (Figure 1). Significant correlation was seen between Optical and mechanical methods for APTT. (r=0.530, p-value=0.000) (Figure 2)

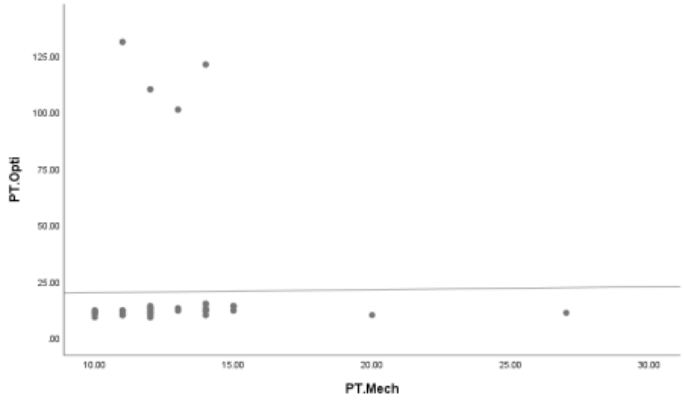

Figure 1. Correlation between Optical method and Mechanical method for PT.

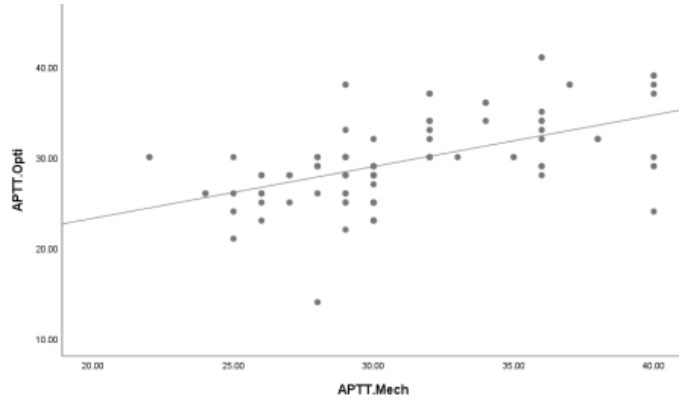

Figure 2. Correlation between Optical method and Mechanical method for APTT.

Discussion

Novel detection techniques are arising to address the limitations of the prior approaches. The coagulometer instrument operates based on two principles: electromechanical and photo-optical. The electromechanical instrument consists of a rotating cuvette made of steel ball with impedance, as well as a rotating steel ball with impedance. Photo-optical techniques encompass various methodologies, including scatter light detection for clotting assays, transmitted light detection for chromogenic assays and immunoassays, nephelometry, photo-optical end point determination and analysis, percentage detection method, and rate method. [10,11]

This research aimed to evaluate the association between photo-optical and mechanical techniques for coagulation testing of prothrombin (PT) and activated partial thromboplastin time (APTT) in samples with turbidity. The results of this investigation indicated a negligible connection (r=0.010, p-value=0.918) for PT, but a statistically significant correlation for APTT (r=0.530, p-value=0.000) using both methodologies.

Bai et al. discovered a strong correlation (R2≥ 0.96) between photo-optical clot detection and electro-mechanical detection systems in all experiments (specifically, R2=0.98). The correlation between the two clot detection methods remained consistent even while assessing turbid samples, with an R2 value of at least 0.98 for all tests, indicating a correlation coefficient of 0.99.8 The investigation found a strong connection between the photo optical and mechanical analysers for PT (R2=0.97, i.e., r = .98), and APTT (R2=0.85, i.e., r=0.92), indicating good agreement. The correlation between the two clot-detection methods remained strong, even while assessing turbid samples. The coefficient of determination (R2) was more than or equal to 0.97, indicating a high level of agreement between the two tests with a correlation coefficient (r) of 0.98.[9]

Our study's results are somewhat consistent with the research described above since APTT shown a strong connection between mechanical and photo-optical techniques. However, there was little association for PT between these two approaches. Pre-analytical mistakes and population variance may be the cause of this. Turbid samples cause erroneously extended findings in photo-optical methods by interfering with light absorption. This highlights how crucial the pre-analytical stage of blood collection is in reducing these disparities.

This research was conducted on a non-pediatric age group whose mean age was 38.83 years. The results revealed that there was little connection between the two techniques for PT, but a strong association for APTT. In our research, 52 of the 100 patients were men and 48 were women. The findings of APTT (strong correlation between photo-optical and mechanical techniques) and PT (poor correlation) did not significantly vary for either gender. Both photo-optical and mechanical clot detection approaches revealed a poor correlation for PT and a positive significant correlation for APTT in 33% of the

smoking patients in this investigation using turbid samples.

According to Kim H research, photo-optical (MTX II) and photo-mechanical analyser (AMAX200) have a strong correlation for both PT and APTT.[9] Strong connection was shown in another investigation by Kaytaz M et al. between electromechanical detection (STA) and photo-optical clot detection technique (SysmexCA-1500) through a foggy sample. [8]

On the other hand, PT and APTT test findings for the lipemic sample cases described by Aggarwal S et al. varied depending on whether coagulometer was used—a photo-optical or mechanical one.[7] In their investigation, Geens T. et al. found that there were notable differences in all metrics, such as PT and APTT, between the Sysmex CS5100 employing optical techniques and the STA-R. This discrepancy in evolution by mechanical means was most likely brought about by variations in techniques and reagents. [12,13,14]

According to research by Avcioglu G et al., the semi-automatic Amax Destiny PlusTM-Trinity Biotech device with an optic-based technique performs better on prothrombin time assessment than the mechanic.[15,16] Our analysis, in which one measure has strong relationship and the other has poor correlation, is consistent with the previously cited findings.

Pre-analytical and analytical factors, such as variations in technique and endpoint detection technologies like photo-optical vs. mechanical clot detection, may cause inter-laboratory distortion in hemostasis testing. [17]Variables related to the reagent and the level of automation in the equipment might affect the outcome.[10] Because blood collection staff members have varying backgrounds and levels of expertise, pre-analytical error resulting in sample integrity for coagulation tests is a developing problem in clinical laboratory practice. In particular, samples that exhibit hemolysis during the blood collection process need to be recognized and, if feasible, substituted with non-hemolyzed samples.

One benefit of photo-optical clot detection is that it may help determine the appropriate coagulation test settings for patients with atypical in-vitro clot responses by combining the analysis of advanced software with a study of the whole clot formation.[18] On the other hand, every mechanical detection system has the drawback of giving the operator just one data point and no way to evaluate it further.[19] It is crucial for labs using mechanical or photo-optical systems to double-check the sample using a different clot detection technique [20] in the unlikely event that a quantifiable end point is not found.

Conclusion

There is a weak correlation (r=0.010, p-value=0.918) between photo-optical and mechanical techniques for PT and a strong correlation (r=0.530, p-value=0.000) for APTT. 2.Updated coagulation test criteria should be used for analysing turbid materials.

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