Original Article

PRE-ANALYTICAL ERRORS IN SAMPLES COLLECTED FOR COAGULATION PROFILE

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ABSTRACT

Objective: To determine the frequency of Pre-analytical errors in sample collected for coagulation profile.
Material and Methods: This descriptive cross-sectional study was conducted at Department of Pathology, Combined Military Hospital Multan Cantt from 14th Oct 2019 to 31st March 2020. We have collected the samples for coagulation profile from Main ITC, CCU, Surgical ITC, Pediatrics, Medical and Gynecology wards and divided these samples into four categories (Cat); Cat 1: Properly filled, Cat 2: Overfilled, Cat 3: Under Filled and Cat 4: Clotted. Data was entered in Microsoft excel for compilation.
Results: Age range was from 1 to 83 years, mean age of 57.4 ± 7.3 years. Among them, 3881 (49.1%) were male and 4026 (50.9%) were females. Out of (7907) samples 6656 samples were properly filled, (927) were over filled, (230) samples was under filled and 94 samples was clotted. In Cat 2, 276 cases (30%), in Cat 3, 36.5% samples showed abnormal results.
Conclusion: Frequency of Pre-analytical variables in sample collected for coagulation profile is quite high.
Key Words: Coagulation, Pre-analytical variables, Prothrombin time

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INTRODUCTION

Since the start of coagulation profiles, struggles have been made to automate/ standardize them to provide accurate test results for effective patient management in a timely fashion [1]. Pre-analytical issues concerning routine coagulation tests can be classified into 3 major categories: (1) collection of specimens (2) transportation and (3) processing and storage of specimen [2]. Among these classes there are numerous variables, which may have impacts on final result, thus may affect patient management. Protocols for testing & procedures in clinical Laboratory (Lab) specifically in coagulation lab have been developed in order to improve accuracy and precision [3]. Clinical Laboratory Standards Institute (CLSI) has observed a deficiency of standardization among clinical laboratories, with respect to specimen/ sample collection, its storage and processing for coagulation testing [4]. As a result of this non-conformances can arise which are based on pre-analytical errors and may be associated with frightening consequences which have been studied in detail by Gosselin RC *et al* [5]. Appropriately trained staff in phlebotomy ensures proper sample collection resulting in better quality of lab results as demonstrated by Stegnar M *et al* [6]. Similarly, there are numerous types of sample collection tubes in market, in which despite similarities of citrate concentration, there are significant differences affecting result. For example, differences in coagulation profile results using different collection tube manufacturers with the same citrate concentration have been reported by Lima-Oliveira G *et al* [7,8].

Sodium citrate (3.2%) is widely used as anticoagulant in coagulation profile. Fill volume of these sample tubes containing sodium citrate and volume of patients plasma is of great concern in coagulation studies. If volume is less or hematocrit (HCT) is significantly elevated (>55%), excess sodium citrate in the sample would potentially inhibit clot formation in test leading to errors [9]. Present-day recommendations dictate that fraction of blood to anticoagulant volume should be 9:1 [10,11,12]. Samples with high HCT (>55%, like in severe dehydration, neonates, burn patients & patients of polycythemia vera etc) may lead to factitiously increased clotting times due to presence of excess citrate in the sample [13]. To address this issue of HCT, Labs may use vacuum tubes with a lesser volume of anticoagulant; for example, by using small gauged needle and removing 20% volume of anticoagulant (without eliminating the vacuum) [13]. In this regard Naz S *et al* have established that Lab workers should adopt a comprehensive approach in

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close coordination with clinicians so as to provide high quality diagnostic services for effective patient management [14].

In view of above, numerous procedural issues, shortcuts/ variations in phlebotomy and implications of inaccurate results, present study was planned to evaluate the frequency of pre-analytical errors in samples collected for coagulation profile.

MATERIAL AND METHODS

A Descriptive, Cross-sectional study was carried out in Department of Pathology, CMH Multan with effect from 14th October 2019 to 31st Mar 2020. Non-probability convenient sampling technique was used for patient selection irrespective of age and genders. We have collected the samples for coagulation profile from Main ITC, CCU, surgical ITC, Pediatrics, Medical and Gynecology wards and divided the samples into four categories; Cat 1: Properly filled, Cat 2: Overfilled, Cat 3: Under Filled and Cat 4: Clotted. A hand on training workshop was conducted by a team of Pathologists for 3 days regarding sample collection to nurses, nursing assistants & phlebotomists. Data was entered in Microsoft Excel for compilation. Frequency & percentage was recoded for sample categories. Mean & SD was calculated for age.

DATA COLLECTION PROCEDURE:

A total of 7907 patients samples for coagulation profile reporting to Department of Pathology in CMH Multan were included in the study after obtaining approval from hospital Ethical Committee. Both outdoor & indoor patients from Main ITC, CCU, Surgical ITC, Pediatrics, Medical and Gynecology wards were included in study. PT tubes with a pre-labelled mark requiring 1.8 ml venous blood in Trisodium citrate tube (200ul) were used for collection of blood and gently mixed at the point of sample collection. Samples from children below 2 years were collected in pediatric tubes. All samples were inspected before analysis for categorization. Samples were centrifuged for at least 15 minutes at 2200-2500 RPM within one hour of sample collection. Coagulation profile including PT, APTT, D-Dimers & Fibrinogen was carried out on these samples

RESULTS

Age range was from 1 to 83 years, mean age of 57.4 ± 7.3 years. Among them, 3881 (49.1%) were male and 4026 (50.9%) were females. Samples for coagulation profile ware categorized as; Cat 1: 6656, Cat 2:927, Cat 3:230 and Cat 4 were 94. Out of 7907 samples, 6656 samples were in Cat 1, 927 in Cat 2, 230 in Cat 3 and 94 samples were in Cat 4. In Cat 2, 276 cases (30%) and in Cat 3, 36.5% samples showed abnormal results. Total tests performed on these samples are shown in Figure1& 2.

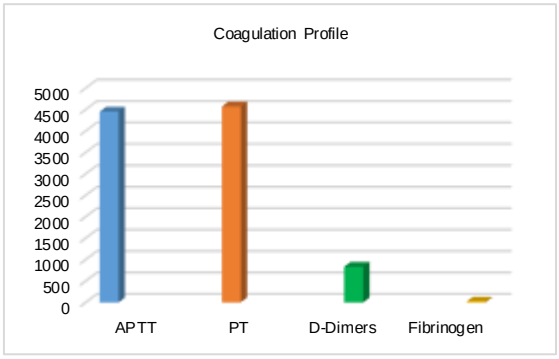

Figure-1: Coagulation profile of samples received in laboratory (n= 7907).

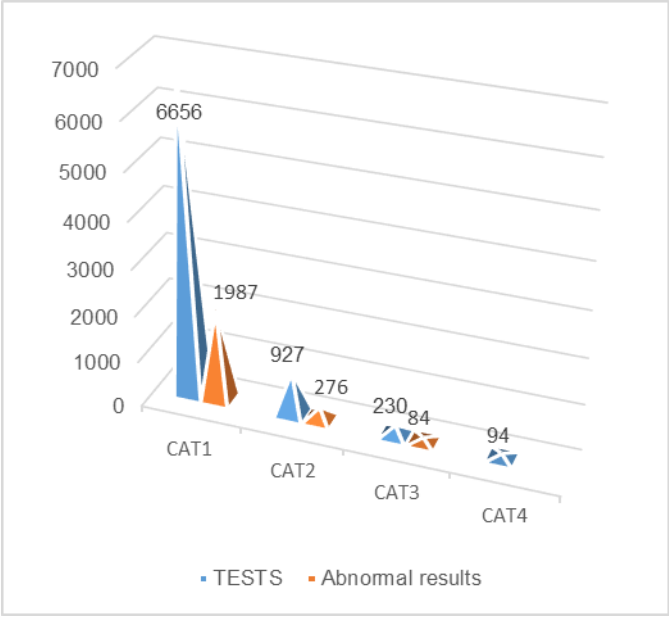

Figure-2: Category wise number of samples received in Lab (n= 7907).

DISCUSSION

Evidence Based Lab Medicine Practice emphasizes that labs must always practice the strictest rules, including those from reagent procurement to collect/ prepare specimens & to standardize testing/ reporting results to improve quality of patient results. As recent advances in science and technology have revolutionized Lab procedures from manual, cumbersome tests to fully automated techniques, ensuring accuracy and speed, therefore diagnosis is heavily dependent upon reliable Lab data, so it is pertinent to emphasize reliability of the results released by clinical labs [15]. In this regard concept of Total Quality Management in Labs (TQM) incorporates all the steps involved in specimen obtaining/ processing, to the final interpretation of results by the clinicians to reduce the

errors/ concerns that may arise during these procedures [16]. Promotion of standardized phlebotomy practices are a pre-requisite for the effective laboratory functioning and finalization of results. Keeping in view the importance of pre-analytical issues, a hands-on training workshop was conducted by a team of Pathologist for 3 days regarding sample collection & transportation to nurses, nursing assistants & phlebotomists at this Hospital [17,18].

Favaloro EJ *et al* have described that Pre-analytical errors in coagulation profile testing were an important basis of mistake and could lead to adverse clinical events. However, the burden of lab errors in their study was modest (i.e., 1 in every 900-2074 patients or every 214-8316 Lab results). Accordingly they concluded that different remedies might support error identification and their categorization including a focused ongoing training of staff regarding types & sources of errors, evaluation of sample quality (i.e. volume, blood to anticoagulant ratio & presence of potential interferents), and the logical recording of doubtful results along with availability of relevant clinical evidence [19]. Similarly, Gosselin RC *et al* pronounced that these errors may be connected with the patient themselves, specimen collection, specimen transportation and specimen processing. Failure to address these pre-analytical variables may result in misappropriation and patient mismanagement [5].

Results of present study show that 1251 (15.8%) samples were overfilled, underfilled or clotted which shows a casual approach towards sample collection. This study was conducted on coagulation profile as it is generally required in critically ill cases or before surgery; results can be misleading on one hand and can misguide clinician in management of patients on other hand as highlighted by Gosselin RC *et al* [5]. Similarly, other researchers have concluded that incongruous Lab tests range from 11- 70% for clinical chemistry & haematology, 5-95% for clinical pathology & microbiology and 17-55% for Immunoassays which are in also accordance with present study [20,21].

In present study out of 7907 samples, 11.7% were overfilled, 2.9% were underfilled and 1.2% samples were clotted. In Cat 2, 276 cases (30%) and in Cat 3, 80 cases (36.5) % samples showed abnormal results which were definitely not accurate as dilutional error would have contributed to final results which has been highlighted by earlier studies [7-12]. These findings are in accordance with Bonini P *et al* who have described the importance of the pre-analytical segment and concluded that misuse of Lab services by requesting inappropriate Lab test had an impact on total costs of patient management, and increased risk of medical errors and outcome of patients [22].

In present study 94 samples were clotted/ hemolysed which reflects improper collection/ handling of sample as studied by Hollensead SC *et al* who have highlighted that insufficient volume was a major cause of rejection of samples in Lab in their study. The main reasons for the discrepancy were; inexperience of the phlebotomist, problematic sampling in certain group of patients as in pediatrics/ neonate patients, debilitated patients, those on chemotherapy and those in whom veins localization was difficult for example morbidly obese patients. They concluded that insufficient quantity of sample was the most frequent reason of rejection which is in accordance with present study too [23]. It has been advocated that samples must be mixed gently by 3 - 6 times by end-over-end tube overturns to ensure adequate mixing of anticoagulant with sample and prevention of clotting/ hemolysis. Insufficient mixing has a bigger effect on coagulation profile performed later than on basic coagulation tests performed sooner after collection. Conversely too hard mixing can lead to in-vitro hemolysis or counterfeit factor activation resulting in falsely low clotting time and even possible false rise of clotting factor activity (e.g. Factor VII) [24]. Similarly, British Committee for Standards in Haematology (BCSH) has recommended manual mixing of sodium citrate containing sample tubes (gently mixing end-over-end the tube 5 to 6 times) to prevent clotting/ hemolysis [10]. At the same time transportation/ handling of blood specimens for coagulation profile includes a critical set of pre-analytic variables which can have a dramatic influence on results, which in turn can have serious concerns for patients [20]. Additionally, advances in laboratory instrumentation/ techniques have improved the quality, reproducibility and sensitivity of the analytical phase, which emphasizes need of sample reliability and other factors in pre-analytical phase to reduce lab errors [25].

CONCLUSION

The frequency of pre-analytical errors in sample collected for coagulation profile is quite high which affects final result of patient.

RECOMMENDATIONS

Continuous training of staff involved in sample collection of samples for laboratory analysis is recommended to ensure accurate and precise results.

AUTHOR CONTRIBUTION

Muhammad Younas: Idea conception, proof reading
Hamid Iqbal: Data collection
Kainat Younas and Muskan Younas: Literature review
Waqas Hanif: Draft preparation
Tahira Rafique: Data analysis

REFERENCES

1. Clinical and Laboratory Standards Institute (CLSI). Available at: https://clsi.org/. Accessed March 09, 2019.
2. International Organization for Standardization (ISO) document ISO 15189: 2012. Medical laboratories-particular requirements for quality and competence. International Organization for Standardization, Geneva, Switzerland.
3. United States Food and Drug Administration (FDA). Bioanalytical method validation guidance for industry. May 2018. Available at: https://www.fda.gov/downloads /Drugs/Guidance Compliance Regulatory Information /Guidances /UCM070107.pdf. Accessed March 2019.
4. Favaloro EJ, Gosselin R, Olson J, Jennings I, Lippi G. Recent initiatives in harmonization of hemostasis practice. Clin Chem Lab Med. 2018; 56(10):1600–9.
5. Gosselin RC, Marlar RA. Preanalytical variables in coagulation testing: Setting the stage for accurate results. Semin Thromb Hemost. 2019; 45:433–8.
6. Stegnar M, Cuderman TV, Bozic M. Evaluation of pre-analytical, demographic, behavioral and metabolic variables on fibrinolysis and haemostasis activation markers utilized to assess hypercoagulability. Clin Chem Lab Med. 2007; 45(01): 40–6.
7. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Picheth G, Guidi GC. Sodium citrate vacuum tubes validation: Preventing preanalytical variability in routine coagulation testing. Blood Coagul Fibrinolysis. 2013; 24(03): 252–5.
8. Raijmakers MT, Menting CH, Vader HL, van der Graaf F. Collection of blood specimens by venipuncture for plasma-based coagulation assays: necessity of a discard tube. Am J Clin Pathol. 2010; 133(2): 333-5.
9. Adcock DM, Kressin DC, Marlar RA. Effect of 3.2% vs 3.8% sodium citrate concentration on routine coagulation testing. Am J Clin Pathol. 1997; 107(01): 105–10.
10. Mackie I, Cooper P, Lawrie A, Kitchen S, Gray E, Laffan M. British Committee for Standards in Haematology. Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. Int J Lab Hematol. 2013; 35(1): 1-13.
11. CLSI. Collection of Diagnostic Venous Blood Specimens. 7th ed. Standard GP41. Wayne, PA: Clinical and Laboratory Standards Institute. 2017.
12. Douxfils J, Ageno W, Samama CM. Laboratory testing in patients treated with direct oral anticoagulants: A practical guide for clinicians. J Thromb Haemost. 2018; 16(2): 209-19.
13. Marlar RA, Potts RM, Marlar AA. Effect on routine and special coagulation testing values of citrate anticoagulant adjustment in patients with high hematocrit values. Am J Clin Pathol. 2006; 126 (3): 400–5.
14. Naz S, Mumtaz A, Sadaruddin A. Preanalytical errors and their impact on tests in clinical laboratory practice. Pak J Med Res. 2012; 1: 27-30.
15. Price CP. Evidence-based laboratory medicine: Supporting decision-making. Clin Chem. 2000; 46 (8): 1041–50.
16. Dayakar S, Pillai HR, Kalpathodi S. A Guide to total quality management system (TQMS) in molecular diagnostics from experiences in seeking accreditation and implementation. SN Compr Clin. 2019:1: 123-33.
17. Tibbets MW, Gomez R, Kannangai R, Sridharan G. Total quality management in clinical virology laboratories. Indian J Med Microbiol. 2006; 24(4): 258–62.
18. Ahmed HAM, Ali LM. Best practices nursing guideline in phlebotomy for patient safety and quality improvement. iOSR J Nursing Health Sci. 2016; (5): 1-16.
19. Favaloro EJ, Dorothy M, Funk A, Lippi G. Pre-analytical variables in coagulation testing associated with diagnostic errors in hemostasis. CE Update Lab Med. 2012; 43: 1-10.
20. Silverstein MD. An approach to medical errors and patient safety in laboratory services. In: A white paper. Atlanta: The Quality Institute Meeting; 2003.
21. Kirchner MJ, Funes VA, Adzet CB. Quality indicators and specifications for key processes in clinical laboratories: a preliminary experience. Clin Chem Lab Med. 2007; 45: 672-7.
22. Bonini P, Plebani M, Ceriotti F. Errors in laboratory medicine. Clin Chem. 2002; 48(2): 69-8.
23. Hollensead SC, Lockwood W, Elin R. Errors in pathology and laboratory medicine: consequences and prevention. J Surg Oncol. 2004; 8: 161-71.
24. CLSI. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture. Approved Standards 6th ed. CLSI document H3-A6. Wayne PA: Clinical and Laboratory Standards Institute. 2007.
25. Price CP, Barnes IC. Laboratory medicine in the United Kingdom: 1948-1998 and beyond. Clin Chem Acta. 2000; 290: 5-36.