Estimation of Coagulation Test, Platelet Count, and Platelet Indices among Polycythemic Patients at Khartoum State - Sudan

Raheeg Hamza M. Hamed[1], Albaraa Abdulfatah Mohammed[2], Ghanem Mohammed Mahjaf[2], Tibyan Abd Almajed Altaher[3], Mosab Nouraldein Mohammed Hamad[4*]

[1]*Department of Haematology and Immunohematology, Faculty of Medical Laboratory Sciences,National University, Sudan.*

[2]*Associate professor, Hematology Department, Alfajr College for science and Technology, Sudan*

[3]*Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Shendi University, Shendi, Sudan.*

[4]*Department of Clinical Chemistry, Faculty of Medical Laboratory Sciences, Shendi University, Sudan.*

[5]*Assistant professor, Microbiology department, Faculty of Medicine, Elsheikh Abdallah Elbadri University, Sudan.*

Corresponding Email: mosab@eaeu.edu.sd

Abstract:

Background: An increase in the total body red cell volume (Polycythemia) directly affects coagulation parameters because of high PCV, the thrombophilic tendency tends toward thrombophilia. ***Aim***: This study aimed to estimate the coagulation profile, platelets count, and platelet indices and correlate the parameter result among polycythemia Sudanese patients. ***Methods***: It was an analytical comparative case-control study conducted in Alzaitona Hospital, Khartoum, from the period January to March 2023 on a total of one hundred venous blood samples collected equally from polycythemia patients (case group) and healthy (control group), then for both group complete haemogram was done using an automated cell counter (SysmexKX-2IN) and coagulation automation (New 2008 STAGO Start4). ***Results***: The result shows normal platelets while there is prolonged prothrombin time and activated partial thromboplastin time. ***Conclusion***: the thrombophilic tendency tends toward thrombophilia among polycythemic patients in Khartoum state.

Keywords: Polycythemic Patients, Coagulation Test, Platelet Count, Sudan.

Introduction

Traditionally, polycythemia has been used to identify a group of varied disorders with increased circulating red cells typified by a persistently raised hematocrit (Hct). Since only the red cell lineage is involved, erythrocytosis has more validity and will be used throughout this article. Polycythaemia will be retained in the clonal disorder, polycythemia vera (PV), in which three cell lineages are involved **[1].** Not much has been learned about the pathophysiology of myeloproliferative diseases, including risk factors for bleeding and thrombosis, despite decades of clinical and laboratory investigation. In polycythemia vera, the pro-thrombotic effect of an elevated hematocrit is well established. On the other hand, essential thrombocythaemia has not been linked to thrombocytosis per se in a similar manner. In both conditions, advanced age and the presence of a prior event identify thrombosis-prone patients **[2].** An absolute erythrocytosis is present when the red cell mass is raised and the hematocrit is elevated above prescribed limits. Causes of absolute erythrocytosis can be primary where there is an intrinsic problem in the bone marrow and secondary where there is an event outside the bone marrow driving erythropoiesis **[3].** Erythropoiesis is a complicated and highly controlled process that starts with a multipotent stem cell in the bone marrow and ends with an enucleated, mature erythrocyte. Inherited bone marrow failure, chronic illness-related anemia, and the direct impairment of medullary erythropoiesis, as observed in thalassemia syndromes, can all lead to altered red cell production. Alternatively, in disorders such as sickle cell disease (SCD) as well as enzymopathies and membrane defects, medullary erythropoiesis is not, or only minimally, directly impaired **[4].** Is a dynamic process; the normal coagulation pathway represents a balance between the pro-coagulant pathway that is responsible for clot formation and the mechanisms that inhibit the same beyond the injury site. An imbalance of the coagulation system may occur in the preoperative period or during critical illness, which may be secondary to numerous factors leading to a tendency of either thrombosis or bleeding **[5].** Two pathways can cause blood to coagulate: the intrinsic system is stimulated by contact with a negatively charged surface, and the extrinsic pathway is triggered by the release of tissue factors from the site of injury. After the first stimulus, a sequence of serine proteases is triggered one after the other, resulting in the synthesis of thrombin, the enzyme that transforms soluble fibrinogen into an insoluble fibrin clot **[6].** The coagulation factors, platelets, fibrinolytic system, and vasculature make up the four compartments that make up the hemostatic system. The intricately controlled interplay between these four compartments cannot yet be replicated in a lab setting or a proximity to a patient. Only the coagulation protein compartment of the system is tested by the prothrombin time (PT) and activated partial thromboplastin time (APTT), and the results must be carefully interpreted in light of the clinical presentation and assay constraints **[7].** Hemostasis is made possible by coagulation, which works in a

complex way using several clotting factors. The components of the intrinsic route include factors I, II, IX, X, XI, and XII. Respectively, each one is named, fibrinogen, prothrombin, Christmas factor, Stuart-Prower factor, plasma thromboplastin, and Hageman factor. The components of the extrinsic route are factors I, II, VII, and X. Stable Factor VII is the name given to it. Factors I, II, V, VIII, and X make up the common route. The factors are activated into serine proteases while moving through the bloodstream as zymogens. By acting as a catalyst, these serine proteases split the subsequent zymogen into further serine proteases, which in turn activate fibrinogen. Serine proteases include factors II, VII, IX, X, XI, and XII. None of the factors VIII, XIII, or V are serine proteases. The intrinsic pathway is activated through exposed endothelial collagen, and the extrinsic pathway is activated through tissue factor released by endothelial cells after external damage **[8].** In addition, several investigators have found a shortened fibrinogen survival in patients with PV, suggesting a state of chronic intravascular coagulation **[9-12].**Conclusion The Hemorrhage and coagulation process in patients with PC could be very complicated, including physiological adaptation and the process of physiology evolving into pathology **[13].**

Materials and methods

Study Design

This study was a case-control descriptive study.

Study area

This study was performed at AL-ZITONA Hospital, Khartoum State, Sudan.

Study period

This study was conducted in the period between January and April 2023.

Study population

The patient who was confirmed diagnosed with polycythemia will be indexed as a case.

Inclusion criteria

A population with polycythemia was enrolled as a case group. Healthy individuals of matched gender, age, and medical history were enrolled as a control group.

Exclusion criteria

Polycythemia patients who recently donated blood (within one week), anti-platelets, anti-coagulant, and thrombolytic drugs, and participated in erythropoietin drugs, were excluded from the study.

Data collection

The data were collected using a pre-designed structural questionnaire, and the demographic and clinical

data concerning each participant were obtained from the registry database office. The laboratory data were obtained from the hematological and coagulation analyses.

Method for Sample Collection

After calculating from a previous study, we found that 100 samples (50 cases and 50 controls) would cover our research in a way that would help us clarify the purpose of the study. Before collection, a local antiseptic (70% alcohol) will be used to disinfect the skin and venous blood. 5 ml will be taken. 5 ml of venous blood was collected from the patient, then divided into 3.2 ml of ethylene diamine tetraacetic acid (EDTA) for platelet counting and 1.8 ml of a sodium citrate anticoagulant tube for coagulation studies. Label with the subject's age, sex, and identification number, then examine in a coagulation analyzer and cell counter.

Method for Complete Cell Count (CBC)

The Principle of Coulter the blood cells with low conductivity are suspended in a liquid conductive diluent. An electric field formed by two electrodes is transferred through the diluent. The liquid flows via a tiny opening. The impedance, or resistance, of the electrical circuit between the electrodes, briefly increases as each particle passes through the aperture. The increase in impedance creates a pulse that can be measured. The number of pulses equals the blood cell count. The amplitude (height) of the pulse is equal to the volume of the cell. After the samples were collected in an EDTA tube, they were used for CBC measurement by an automated cell counter (Sysmex KX-2IN).

Method for coagulation (PT, APTT)

The electromagnetic field uses a magnet in the test tube aligned with the magnetic detector in the cuvette and remains locked in position with the detector while the test tube rotates. a clot form, it entangles (confuses) the magnet, breaking the electromagnetic coupling and allowing the magnet to rotate with a tube, terminating the test. After the samples were collected in sodium citrate anticoagulant, they should be placed in a centrifuge at 3000–4000 rounds per minute for 15 minutes. They were used for PT and APTT (New 2008 STAGO Start 4). Within the limits of observing quality control and making sure of the automation and pipetting the cuvette in the incubation area, then a magnetic ball was put in each of them, an activated test mood was displayed on the screen, and PT and APTT entered the test numbers. 50 microliters of PPP were added, then incubated at 37 °C in two tubes. 50 microliters of prewarmed APTT reagent (Koalin, cephalin mixer Biomed, Egypt) were incubated for 2–3 min, then 50 microliters of calcium chloride and 100 microliters of PT reagent (thromboplastin) were added after incubating the sample for 1 min.

Data analysis

The data obtained through the study were entered and analyzed by computer using Microsoft Office

Excel and the SPSS statistical program to calculate the mean, standard deviation, and P-value by t-test. P<0.05 was considered statistically significant.

Ethical consideration

The study was released to the National University Research Committee, and then written consent was obtained from each patient before sample collection.

Results

A total of hundred (100) participants were enrolled in this study, 50 were selected as cases and 50 were selected as a control group. Samples were collected in Khartoum state in AL-ZITONA Hospital between January 2023 and April 2023. According to age were 20-30 years (35, 70%), and 31-40 years (15, 30%). Hemoglobin levels among case groups were distributed as 50 frequencies, (17-18g/dl) (19,38%),(18.1-19),(11,22%),(19.1-20)(12,24%),(20.1-21)(4,8%),(21.1-22) (4,8%) and control 50 frequency, (14)(8,16%),(15)(24,48%), (16)(12,24%), (17)(6,12%) **(Table 1).** The Hemoglobin was significantly increased among the case group compared to the control (mean 18.8) for case and control respectively (*P. value* < 0. 05) **(Table 8).** The PT mean was significantly prolonged among the case group compared to the control (14.7) for case and control respectively (r=0.780 *P. value*> 0.05) **(Tables 2, 8, and 9).** The APTT mean was significantly prolonged among the case group compared to the control (36.0) for case and control respectively (r=0458 *P. value* >0.05) **(Tables 3,8 and 9).** The PLT count showed an insignificant decrease among the case group compared to the control mean (189.8) for case and control respectively (r=-0.268 *P. value* <0.05) **(Tables 4,8 and 9).** PCV was a significant increase among the case group compared to the control (52.2) for case and control respectively *P. value* (0.000) **(Table 8).** MPV was a significant increase among the case group compared to the control (8.4) for case and control respectively *P. value* (0.000) **(Tables 5 and 8).** PDW was insignificant among the case group compared to the control 50(15.4±0.53) for case and control respectively *P. value* (0.000) **(Tables 6 and 8).** PCT was insignificant among the case group compared to the control 50(0.15±0.023) for case and control respectively *P. value* (0.000) **(Tables 7 and 8).** The study found that the highest frequency was 24(48%) in which group (18.1-19) and the lowest on 6 (12%) in the age group (21.1-22) years.

Table 1: Frequency Hb case and control

t		*N (%)*	*rol N (%)*	
	8)	(38%)		%)
	-19)	(22%)		3%)
	-20)	(24%)		-%)
	-21)	(8%)		%)
	-22)	(8%)		
		00.0%)	0.0%)	

Table 2: Frequency PT case and control

t		*N (%)*	*Control N (%)*	
		-4%)		%)
		46%)		-%)
		0%)		
		0.0%)	0.0%)	

Table 3: Frequency APTT case and control

t		*N (%)*	*Control N (%)*	
	)	80%)	)	%)
	)	20%)	)	%)
		0.0%)	0.0%)	

Table 4: Frequency PLT case and control

t		*N (%)*	*Control N (%)*	
	90	%)	90	)
	30	%)	30	%)
	70	)	70	%)
	10	)	10	%)
	50	%)	50	%)
		0.0%)	0.0%)	

Table 5: Frequency MPV case and control

t		*N (%)*	*Control N (%)*	
		%) 2%) %)		%) .%) %)
		0.0%)	0.0%)	

Table 6: Frequency PDW case and control

t		*N (%)*	*Control N (%)*	
		) %) %)		%) %)
		0.0%)	0.0%)	

Table 7: Frequency of PCT case and control

t		*N (%)*	*Control N (%)*	
	-0.16)% -0.23)%	%) %)	-0.16)% -0.23)%	%) %)
		0.0%)	0.0%)	

Table 8: Compare platelet and their indices with case and control

ble	*N(Mean±SD)*	*ol N(Mean±SD)*	*lue*
	3±0.47)	42±0.5)	
	.7±0.7)	.44±0.5)	
T	.0±3.5)	.5±2.5)	
	.8±1.4)	.3±0.9)	
	.2±4.4)	.6±2.7)	
	9.8±28.1)	9.9±45.1)	
	4±0.70)	9±0.5)	
	.4±0.53)	.7±0.48)	
	15±0.023)	19±0.03)	

Table 9: Correlation between Hb and PT, APTT and PLT

		lue		*lue*		*lue*
					3	

Discussion

Traditionally, polycythemia has been used to identify a group of varied disorders with increased circulating red cells typified by a persistently raised hematocrit (Hct). Since only the red cell lineage is involved, erythrocytosis has more validity and will be used throughout this article. Polycythaemia will be retained about the clonal disorder, polycythemia vera (PV), in which three cell lineages are involved. A total of hundred (100) participants were enrolled in this study, 50 were selected as cases and 50 were selected as a control group. Were collected studies in Khartoum state In AL-ZITONA Hospital between January and April 2023 the homological clinic diagram with polycythemia will be indices as a case. All patients with Polycythemia in this study had a high level of Hb case group (17-18) (19,38%),(18.1-19),(11,22%),(19.1-20)(12,24%),(20.1-21)(4,8%),(21.1-22)(4,8%) compared the control group (14) (8,16%), (15)(24,48%), (16)(12,24%), (17)(6,12%). And PT with a case 14(22,44%), 15(23,46%), and 16 (5,10%) compared the control group 11(28,56%), 12(22,44%), APTT a match between case and control to normal range (24-30)(40,80%) and (31-35)(10,20%), PLT a match between case and control to normal range (150-190) (31, 62%) control (4, 8%) (191-230)(15,30%%) control (14,28%), (231-270)(3,6%) and control (13,26%), (271-310) (1,2%)and control (14,28%), (311-350) (0,0%) and control (5,10%). The study showed that there is an increase in the mean levels of Hb (18.8±1.4) and PT (14.7±0.7), APTT (36.0±3.5), PCV (52.2±4.4) compared to the control group Hb (15.3±0.9), PT (11.44±0.5), PCV (52.2±4.4), with the significance of (*P. value* 0.000) offset by a significant decrease in the mean levels of PLT (189.8±28.1), compared the control group PLT (249.9±45.1) this is studied consistently with the study Zhongguo Guzhi Shusong Zazhi study. It also showed that there is a negative correlation between Hb and PLT(r=-268)with a significance of 0.06 and it relatively matches the study Zhang, Xiaochuan Yu, Yuanzhen Shen, Chunhui and a positive correlation between high Hb and PT and APTT(r= 780),(r=0.458)with significance of 0.000, 0.0001 respectively. This change in coagulation parameter is likely due to cell expansion that occurs in polycythemia which affects the amount of plasma protein.

Conclusion

According to the study PT, APT was significantly increased and non-significant of PLT and their indices.

Recommendations

Based on the previous study the following recommendations are suggest periodic follow-up for this patient, to conduct molecular techniques to detect mutation in this category.

Sources of Funding

There was no specific grant for this research from any funding organization in the public, private, or nonprofit sectors.

Conflict of Interest

The author has affirmed that there are no conflicting interests.

References

1. M. F. McMullin International Journal of Laboratory Hematology29 October 2008 ، Volume 30, Issue 6 p. 447-459.
2. Acquaye C, Blanchette-Mackie EJ, Reindorf C, Edelstein S, Schechter AN. Electron microscopic studies of the intracellular polymerization of sickle hemoglobin. Blood Cells. 1988;13:359.
3. Mary F McMullin, D Bareford, P Campbell, AR Green, Claire Harrison, Beverley Hunt, D Oscier, MI Polkey, JT Reilly, E Rosenthal, Kate Ryan, TC Pearson, Bridget Wilkins, General Haematology Task Force of the British Committee for Standards in Haematology British Journal of Haematology 130 (2), 174-195, 2005
4. Thornton P, Douglas J. Coagulation in pregnancy. Best Pract Res Clin Obstet Gynaecol. 2010;24:339–52
5. Lucy A Norris. Best Pract Res Clin Obstet Gynaecol. 2003 Jun;17(3):369-83. doi: 10.1016/s1521-6934(03)00014-2.
6. R Coll Physicians Edinb ,2014;44(1):42-5.doi: 10.4997/JRCPE.2014.110
7. Chaudhry R1, Usama SM2, Babiker HM3. Treasure Island (FL), 01 Mar 2018 PMID: 29489185
8. Tytgat GW, Collen D, Vermylen J Br J Haematol, 22 (1972), p. 701.
9. Christensen LE, Acta Med Scand, 162 (1958), p. 407.
10. Blömback LA, Carlson LA, Franzen S, Zetterquist E, Acta Med Scand, 179 (1966), p. 557.
11. Martinez J, Shapiro SS, Holburn RR, Blood, 42 (1973), p. 34.

12. Zhang, Xiaochuan Yu, Yuanzhen Shen, Chunhui Yang, Fengjuan Liu, Shengliang Ye, Xi Du, Li Ma, Haijun Cao, Zongkui Wang, Changqing Li Hematology 24 (1), 325-330, 2019

13. Chinese Journal of Osteoporosis / Zhongguo Guzhi Shusong Zazhi. Feb2020, Vol. 26 Issue 2, p294-297. 4p.

14. Francesco Passamonti et al. Am J Med. 2004 Nov 15;117(10):755-61.doi: 10.1016/j.amjmed.2004.06.032.

15. Zhongguoshi yan xue ye xue za zhi, 01 Jun 2022, 30(3):813-818 DOI: 10.19746/j.cnki.issn.1009-2137.2022.03.024 PMID: 35680810.