

Basophil Count as a Predictor to the Deep Vein Thrombosis and the Thromboembolization

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Abstract

Basophil is one of the granulocytes leukocytes that has many functions, one of them is the secretion of heparin. The aim study is investigated the correlate the basophilic count with the low endogenous heparin and thrombosis in the deep veins and the thromboembolism. A cross-sectional study conducted at Al Sader Medical City (radiology unit, Doppler unit study and emergency room) during the period from December 2015 to February 2017 included 203 patients with diagnosis of deep vein thrombosis (DVT) by Ultrasonic Doppler study or pulmonary embolism (PE) by CT pulmonary angiography. A group of 205 subjects without evidence of DVT were used as a control. One hundred sixty-seven patients (82.3%) had DVT for first time, while 14 (6.9%) had recurrent DVT, and 22 (10.8%) had PE. The total male number has thromboembolism disease 52 but total female number is 151 with a male to female ratio was 1: 2.9. At current study founded the basophil percent and basophil count in patient with thromboembolism disease was decreased than normal value, The mean basophil percent in thromboembolism disease was $0.35\% \pm 0.28\%$ while mean basophil count is 30 ± 26 , It had been significantly found that basophil percent and basophilic cell count had a strong association with thromboembolism. Basophil percent had a sensitivity of 82%, specificity 79.5% and accuracy of 80.8%. The corresponding values of these parameters for the basophil count were 85%, 73.2% and 79.1%. In conclusion; both low basophil percent and the basophilic count are significantly associated with DVT, recurrent DVT and PE.

Keywords: Basophil; Thrombosis; Pulmonary angiography

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Introduction

DVT is a common condition affecting 1-2% of the population¹ and the case mortality is 1–3%. It is increasingly common with ageing.² DVT can lead to death through pulmonary embolism (PE) and many patients subsequently suffer from venous reflux, which can lead to the post-thrombotic syndrome (PTS).³(80%) of pulmonary emboli arise from the

propagation of lower limb DVT. It occurs in approximately 1% of all patients admitted to the hospital. There are 100,000 to 180,000 deaths annually from PE among hospitalized patients.⁴ Endogenous heparin a mucopolysaccharide molecule produced in the liver lung and aorta by mast cell, endothelial cell and basophil.⁵ Endogenous heparin augments anticoagulation activity by binding to antithrombin (AT). It also contributes to the bleeding time in humans and enhanced blood loss from the microvasculature by its interaction with platelets and endothelial cells, and increased fibrinolysis by its actions.⁵ Aims of the Study: To evaluate the relationship between deep vein thrombosis, thromboembolization and basophil cell count.

Patients and Methods

Study design: A cross-sectional study conducted at Al Sader medical city in Al Najaf city, radiology unit "Doppler unit study" and emergency room during the period from December 2015 to February 2017. A total of 203 subjects with DVT and PE were included in this study and 205 subjects with no DVT used as a control group.

Ethics consideration: Patients agreement with written consent was obtained after explaining the aim of the study and agreement of hospital health administration.

Inclusion criteria: Patients with DVT and PE, who were agreed to participate in the study, regardless of their age or gender.

Exclusion criteria:

Patients with atopy or on steroid therapy. Patients with a myeloproliferative disorder that been discovered by the hematological test as well as patients with a thrombocytosis "platelet count more than $450 \times 10^9 /L$ ", polycythemia "hemoglobin more than 160 g/L in female and 170g/L in male". Patient with leukopenia "WBC count <40000 cells/L ".or leukocytosis WBC >10000 cells per/L thyrotoxicosis Women with the abnormal menstrual cycle or Pregnant

Case-control: 205 subjects were used as a control group

Methods

Demographic data: Including age, gender, and history of the previous same condition. Weight and height of the patients were measured and the body mass index was calculated

Hematological assay: A sample of venous blood was taken from each patient to measure WBC count, hemoglobin, platelet count and basophilic count as well as basophil percent .by Cell-Dyn “Ruby” Laboratory machine

Ultrasonic Doppler study examination: Using B mode ultrasound 7.5 MHz transducer (GE-LOGIC). For a normal vein when imaged in cross-section, it readily collapses with gentle manual pressure from the ultrasound transducer. In the setting of acute DVT, there is dilated main deep veins and loss of venous compressibility by applying a pressure due to the effect of thrombus. The diagnosis of acute DVT is even more secure when the thrombus is directly visualized by B mode. It appears homogeneous and has low echogenicity. Then, using color doppler for the examination of these veins, it showed no color filling of the thrombosed veins. (Figure 2.1)

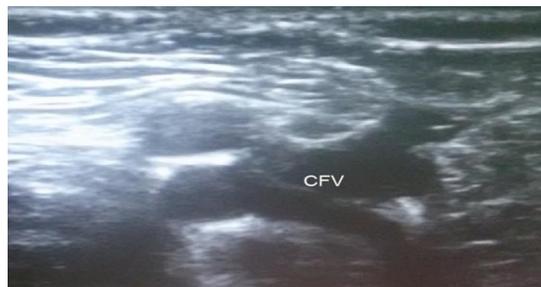


Figure 2.1 Venous ultrasound with compression of the leg veins; CFV . commen femoral vein .

CT pulmonary angiography: Examining patients in supine position after injection of 50 ml of iodinated contrast media with concentration 350mg/ml. The patient hold breath during the examination and the study start by automatic triggering when the pulmonary artery density reaches 180HU and with using Medrad Stellant CT pulmonary angiography as a diagnostic test for patients. The PE diagnosis depends on the visualization of an intraluminal filling defect in the pulmonary arteries in more than one projection. Secondary signs of PE include abrupt occlusion of vessels, segmental Oligemia or Avascularity, a prolonged arterial phase with slow filling, and tortuous, tapering peripheral vessels.

Statistical analysis

Data of all patients and controls were entered and analyzed using the Statistical Package for Social Sciences (SPSS), version 24, IBM, USA, 2013. Descriptive statistics were presented as mean, standard deviation (SD), frequencies (No.) and proportions (%) Receiver Operating Characteristics (ROC) curve analysis was used to assess the validity of Basophil count and percent as predictors of thromboembolism. The area under the curve (AUC) was calculated, (ranger 0 – 100%), AUC more than 50% (0.5) indicates the ability of a test to predict the outcome, the larger AUC the test, the stronger prediction. Level of significance P Value of ≤ 0.05 considered as significant. Finally, the results and findings presented in tables and figures with an explanatory paragraph for each, using the Microsoft word 2010 software for windows.

Results

Total of 203 patients with thrombosis was enrolled in this study. The thromboembolism disease was divided into three groups as DVT, recurrent DVT, and PE. The total number of patients for each group was 167, 14, 22 respectively. Males distribution in each were 38, 5, 9 respectively and females were 129, 9, 13 respectively; that compared with control "group patients "cataract patients" were 56 males with 149 females "Table 3.1.

Table 1.

Gender distribution of the studied groups

	Male		Female		Total
	no.	%	no.	%	
DVT	38	22.8%	129	77.2%	167
DVT (recurrent)	5	35.7%	9	64.3%	14
PE	9	40.9%	13	59.1%	22
Total	52	25.6%	151	74.4%	203
Control	56	27.3%	149	72.7%	205
P = 0.24					

The total male number with thromboembolism disease were 52 patients while female number were 151 patients with a male to female ratio of 1: 2.9; those comparison with control patient "Table .2"

Table 2.

Gender distribution of the thromboembolism and control groups

	Male	Female	Total

	No.	%	No.	%	
Thromboembolism	52	25.6%	151	74.4%	203
Control	56	27.3%	149	72.7%	205
P = 0.70					

Table .3 shows basophil percent and basophil count in thromboembolic disease comparison with control patients that mean basophil percent at thromboembolism disease was $0.35\% \pm 0.28\%$ while mean basophil count is 30 ± 26 but at control group is $0.85\% \pm 0.35\%$, 72 ± 38 respectively that is significant in thromboembolism disease.

Table 3.

Comparison in basophil percent and count of the thromboembolism and control groups

	Basophil percent	Basophil Count
	<i>Mean \pm SD</i>	<i>Mean \pm SD</i>
Thromboembolism	$0.35\% \pm 0.28\%$	30 ± 26
Control	$0.85\% \pm 0.35\%$	72 ± 38
P Value	< 0.001	< 0.001

Table.4 show comparison in Basophil percent and count of the studied groups which is more significant in recurrent DVT than in DVT and it was least in PE comparison with the control group.

Table 4.

Comparison in basophil percent and count of the studied groups and Multiple comparison (ANOVA), P. values

	Basophil percent		Basophil Count	
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
DVT	0.32%	0.25%	28	23
DVT (recurrent)	0.27%	0.22%	25	22
PE	0.60%	0.40%	50	38
Control	0.85%	0.35%	72	38

Multiple comparison (ANOVA), P. values

DVT vs. DVT (recurrent)	0.54	0.78
DVT vs. PE	< 0.001	0.002
DVT. vs. control	< 0.001	< 0.001

DVT (recurrent) vs. control	< 0.001	< 0.001
DVT (recurrent) vs. PE	0.002	0.002
PE vs. control	< 0.001	0.003

Figure 1, demonstrates the Receiver Operating Characteristics (ROC) curve analysis for the validity of Basophil count and percent as predictors of Thromboembolism, it had been significantly found that Basophil percent and count were strong predictors of thromboembolism; area under the curve (AUC) was 0.864 and 0.840, respectively (P<0.001), on the other hand, depending on the ROC curve findings, Basophil percent had a sensitivity of 82%, specificity 79.5% and accuracy of 80.8%.

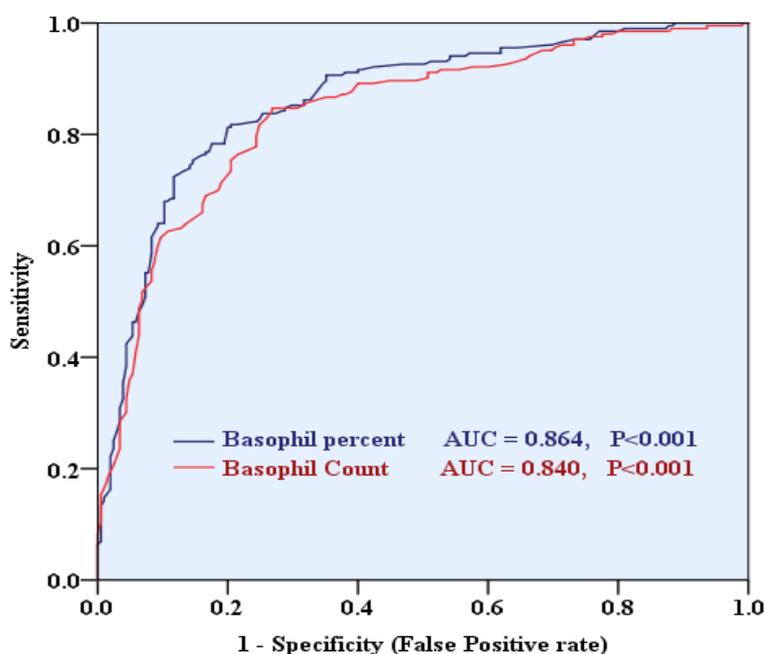


Figure 1.

Receiver Operating Characteristics (ROC) Curve for the validity of basophil count and percent as predictors of thromboembolism.

The corresponding values of these parameters for the basophil count were 85%, 73.2% and 79.1% which indicated that the basophil percent was relatively more specific and more accurate compared to count, which was relatively more sensitive, (Table 5).

Table 5 Validity parameters of basophil count and percent for the prediction of thromboembolism

Validity parameter	Basophil Count	Basophil Percent
Sensitivity	85.0%	82.0%

<i>Specificity</i>	73.2%	79.5%
<i>Accuracy</i>	79.1%	80.8%
<i>PPV</i>	76.0%	80.0%
<i>NPV</i>	83.0%	81.5%

Discussion

Basophil has the least number of the leukocytes, still, it has many functions, one of them is the secretion of heparin. The current study tried to find any association between the basophil cells number and hence their endogenous heparin secretion with common thrombotic problems namely DVT and the pulmonary embolization. The current study showed a clear and significant relation between the low basophil count as well as the basophilic percent in the blood picture with common thrombotic problems namely the DVT .recurrent DVT and the pulmonary embolism. In our study we excluded cases that are known to increase the total leukocytes number as it may cause more basophil count as well as cases that are associated with increase in the basophil count namely those of allergy and myeloproliferative disorders as it was found in limited myeloproliferative disorder cases like Polycythaemia Vera and essential thrombocythemia an increase in DVT occurrence ^(6,7). It was found that the risk of thrombosis is high when the leukocytes number exceed 15000 and also a risk of more thrombosis in cases in early profibrotic myelofibrosis ^(8,9). The increase in leukocytes in cancer cases was also found to be associated with more thrombosis and thromboembolization and may increase the mortality in those patients⁽¹⁰⁾. Few old studies concerned with the basophil and thrombotic events. Ernst et al early in 1972 tried to evaluate the monocytes and basophil numbers in blood samples which were taken from internal jugular veins, external jugular veins and ear lobes in 30 patients with the cerebrovascular attack, the study correlated the monocytes level to the brain pathological stroke and the low level of the basophil was correlated to the low endogenous heparin, while neutrophil and eosinophil level did not show differences . These findings were discussed focusing on the role of monocytes as precursors of cerebral macrophages and of basophils as carriers of endogenous heparin, the examination of ear lobe blood in the study was regarded as a noninvasive and simple method for hematological studies of cerebral disorders. Patients in the study with bilateral brain lesions had fewer basophils in ear lobe samples than normal controls but showed no side-related difference. When a single

intravenous injection of 5,000 units of heparin caused an existing bias in basophil and monocyte values between focal and opposite side to disappear and produced an increase in the cranial level of these cells. The side-related differences did not reappear for an average of three weeks⁽¹¹⁾. Hyman Engerberd et al reviewed published studies since 1995 dealing with many atherogenic mechanisms where exogenous heparin was used with beneficial effect. while endogenous heparin deficiency was found to cause more atherogenesis. Mechanisms included inflammatory factors, lower endogenous plasma heparin levels, and decrease in lipoprotein lipase activity. The reduction of plasma endogenous heparin (heparan sulfate proteoglycans) (HSPG) which had its effect on the lipoprotein activity and the lipoprotein lipase which affects the lipid metabolism found to cause more atheroma in blood vessels⁽¹²⁾. It is well observed that pregnant and lactating women to have more chance to develop venous thrombosis .and it was also observed that healthy women with normal pregnancy had elevated was also observed that healthy women and normal pregnancy had elevated leukocytes and decrease basophil cell count⁽¹³⁾. The last event related with the number of basophil and other leukocytes level was studied in lactating rabbits with thrombotic events and when they were followed for 6 weeks postpartum, the neutrophils found to increase while basophils eosinophils and lymphocytes decreased⁽¹⁴⁾. This low basophilic count may reflect low endogenous heparin and with other factors may contribute to the frequent DVT and pulmonary embolization in pregnancy and lactation. It was found in early studies that after three days of exogenous heparin intake the basophilic count reduced⁽¹⁵⁾ and this may reflect the regulation between the endogenous and exogenous heparin level. Earlier study found that patients with migraine headache showed less than normal blood basophil and less normal endogenous heparin and uroheparin level and the patients who complain from migraine showed clinical improvement when they were treated with heparin with a stable level of the basophil and the uroheparin⁽¹⁶⁾. Heparan sulfate proteoglycan (the endogenous heparin) that secreted from the mast and basophil) can be estimated in the blood and was studied to see its impact on the thrombosis. The heparin works through the antithrombin which was found that partial deficiency of it may be associated with significant thrombosis. The effect of endogenous heparin as antithrombotic was evaluated when it was found to protect cultured arterial endothelial cells from the damage of toxic oxygen metabolites. Toxic oxygen metabolites can damage endothelial cells and can play an important role in the start and the progression of atherosclerotic lesions⁽¹⁷⁾. Leukocytes recruitment to tissues is an important component of inflammatory and immune responses to the injury. However, the excess of leukocytes into sites of inflammation is crucial for the pathogenesis of several inflammatory conditions. Leukocyte rolling on activated endothelium is mainly mediated by selectin molecules. Some

heparin-like glycan can inhibit selectin-mediated leukocyte interaction with platelets and Heparan Sulfate (the endogenous heparin) found to have anti-inflammatory effects probably mediated by selectins, and this may indicate that the glycan may be a potential alternative to heparin for blocking inflammatory reactions without any bleeding effect⁽¹⁸⁾ Endogenous heparin found on the negatively charged luminal surfaces of the endothelial cells is essential for inhibition of coagulation at the endothelial surface and lipoprotein lipase function, both of which are defective in atherosclerotic vascular endothelium. Circulating endogenous heparin and heparan sulfates in the proteoglycan layer of the intima subjacent to the lumen bind with and activate antithrombin to form an effective anticoagulant complex on the endothelial surface. Endogenous heparin has been shown to decrease microthrombi formation at sites of endothelial injury. ⁽¹⁹⁾ While many studies concerned with the Mast cells as they secrete the heparin, limited studies were concerned with the basophil function. It was just for the last few decades when the scientists put differences between the two cells as both secretes the heparin and histamine and share the same function in allergy and in immunity. ...A study to evaluate the role of mast cells (MCs) in DVT initiation and validate MCs as a potential target for DVT prevention. When ligation of inferior vena cava(IVC) was done to create DVT, it was found that degranulation of mast cells in the IVC walls was associated with thrombosis. MCs exacerbate DVT likely through endothelial activation and Weibel–Palade body release, which is, at least in part, mediated by histamine. It was concluded that MCs although they do not directly contribute to normal hemostasis, they can be considered potential targets for the prevention of DVT in humans. ⁽²⁰⁾ . Vance G.Nielsen 2001 showed the importance of endogenous heparin in decreasing the thrombotic event in hemorrhagic shock in experimental rabbit ⁽²¹⁾The decreased level of endogenous heparin was also noted in peripheral occlusive arterial disease which was well correlated with the disease severity ⁽²²⁾. It was observed in many studies that DVT is associated with increased in leukocytes and elevation in CRP, all reflect the inflammatory response that associate the thrombosis. The last fact also observed in patients with acute myocardial infarction and was considered as one of the risks associated factor both in STEMI and non-STEMI ^(23,24). Although the level of neutrophil increases the basophilic count was low in cases of acute myocardial infarction ^(25, 26). In the current study, the basophil cell percentage was more specific and accurate (80.8%accuracy in detecting thrombosis) when compared to basophils cell count which was relatively more sensitive.

Conclusion

Low Basophil percent or count is significant and can predict DVT, recurrent DVT or PE patients. The basophilic percent is relatively more specific as a predictor of the thrombosis.

Further studies with measurement of endogenous heparin with basophil count are highly suggested.

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