Soluble P-selectin Level in Patients with Deep Venous Thrombosis

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ABSTRACT

Background: Venous thromboembolic disease (VTE) remains a significant source of morbidity and mortality. As non-specific subjective complaints and a lack of objective clinical examination findings complicate the diagnosis of both deep venous thrombosis (DVT) and pulmonary embolism. Objective diagnostic testing is required to confirm or exclude the presence of venous thromboembolism before subjecting patients to unnecessary long-term anticoagulation. **Aim of the Work:** This study was aimed to measure the level of soluble P-selectin (sPsel) in Egyptian patients with DVT and asses its diagnostic value in relation to other clinical data and radiological examination. **Subjects and Methods:** This study was carried out on 80 individuals, attending Ain Shams University Hospitals between October 2015 and March 2016 after Ethical committee approval. They were divided into two groups.: **Group I,** 50 patients who were positive for DVT by duplex ultrasound. They were 41 males and 9 females. Their ages ranged 20 - 77 years, with a mean of 46.20 ± 11.78 years. They were 41 (82%) males and 9 (18%) females, with a male to female ratio of 4.55:1. **Group II:** 30 healthy subjects with no clinical signs, symptoms, or history of DVT. They were 23 (76.7%) males and 7 (23.3%) females, with a male to female ratio of 3.28:1. **Results:** There was a highly significant difference between patients (group I) and controls (group II) as regards P-selectin, the best cut off was70.5 ng/ml with 98% sensitivity, 100% specificity, a NPV of 96.8% and a PPV of 100%.
Conclusion: Diagnostic cut-off levels of P-selectin in cases with DVT is 70.5 mg/L that can safely differentiate patients who are free from DVT from others who are positive for DVT or those who would eventually develop thrombosis regardless their primary duplex ultrasound scanning results. **Recommendations:** As our study recommends the use of serum P-selectin as diagnostic biomarker in DVT alone; in addition to the newly estimated cut-off levels for these biomarker, further studies on larger number of cases are needed for more evaluation of these cut-off values and to establish whether they could be used to guide anticoagulation therapy when duplex ultrasound is unavailable.
Keywords: P-selectin, DVT, VTE, clinical data, radiological examination.

INTRODUCTION

Venous thromboembolism (VTE) remains a significant health problem of which Deep vein thrombosis (DVT) is a common life-threatening disorder, affecting approximately 1-3 per 1,000 of the population each year [(1)].

Deep vein thrombosis (DVT) usually refers to the formation of a thrombus in the deep veins of the leg, although DVT may also occur in the veins of the upper limbs. DVT can occur spontaneously without a known underlying cause or after provoking events, such as trauma, cancer, surgery or acute illness [(2)].

Timely and accurate diagnosis of DVT is often difficult due to the diffuse symptoms a patient may manifest. Currently, scoring systems based on a patient's presentation are used to establish the probability of having a venous thrombosis and to determine if further diagnostic testing is warranted [(3)].

In the majority of cases, diagnosis is based upon confirmatory compression duplex ultrasound. However, ultrasound is not always available. Use of plasma D-dimer testing has proved successful in excluding the presence of venous thrombosis. However, there is no current biomarker or combination of biomarkers and clinical presentation that can confirm the diagnosis, when ultrasound is unavailable [(4)].

In the 1970's, Gwendolyn J. Stewart suggested a relationship between inflammation and thrombosis [(5)].

Several studies, despite the small sample size, have demonstrated elevated levels of soluble P-selectin (sPsel) in patients with deep venous thrombosis [(7)].

P-selectin, a protein from the lectin family and a cell adhesion molecule is the first up-regulated glycoprotein on activated endothelial cells and platelets and has procoagulant properties. P-selectin, stored in the platelets (alpha granules) and in the endothelial cells (Weibel-Palade bodies), is translocated to the cell surface after activation and partially released into the circulation in its soluble form. The binding of P-selectin to its specific counter-receptor, P-selectin specific ligand-1 (PSGL-1, present on the surface of leukocytes and platelets), initiates various procoagulant mechanisms [(8)].

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This study was aimed to measure the level of soluble P-selectin (sPsel) in Egyptian patients with DVT and asses its diagnostic value in relation to other clinical data and radiological examination.

SUBJECTS AND METHODS

This study was carried out on 80 individuals, attending Ain Shams University Hospitals between October 2015 and March 2016 after Ethical committe approval. Informed consent was obtained from patients to use their samples. They were divided into:

Group I:

This group included 50 patients who were positive for DVT by duplex ultrasound. They were 41 males and 9 females. Their ages ranged from 20 - 77 years, with a mean of 46.20 ± 11.78 years. They were 41 (82%) males and 9 (18%) females, with a male to female ratio of 4.55:1.

Group II:

This group included 30 healthy subjects with no clinical signs, symptoms or history of DVT. They were 23 (76.7%) males and 7 (23.3%) females, with a male to female ratio of 3.28:1.

Their ages ranged from 20 - 66 years, with a mean of 44.56 ± 11.66 years.

The patients fulfilled the following inclusion criteria:

- Age 18 years or over.
- Confirmed diagnosis of DVT by duplex ultrasound imaging.

Patients were subjected to the following:

A- Full clinical history with special stress on smoking, obesity, medications especially oral contraceptive pills, personal or family history of DVT, concurrent medical problems, history of cancer, serious extremity injuries and history of recent surgery or bedridden > 3 days.

B- Thorough clinical examination laying stress on symptoms and signs of thrombosis.

C- Duplex ultrasound examination of the affected extremity.

D- Laboratory investigations including:

1. Complete blood count (CBC) using Beckman Coulter counter (Coulter Corporation, Florida, USA).
2. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) using Sysmex CA-1500 (Siemens Diagnostics – Germany) [(9)].
3. CRP analysis by Rapid latex agglutination test for the qualitative screening and semi-quantitative determination of C-reactive protein (CRP) in serum (Biomed- CRP– Germany) [(10)].
4. Measuring the level of sPsel in the blood, via an Enzyme Linked Immune-Sorbent assay (ELISA) [(11)].

Sample Collection:

For each patient and control subject, of venous blood were withdrawn under complete aseptic conditions using vacutainer test tubes. Blood samples were divided as follows:

- 2 mL blood were put in a tube containing potassium ethylene diamine tetra-acetic acid (K2 – EDTA) as an anticoagulant in a concentration of 1.2 mg/ml for CBC.
- 2 mL of the remaining blood were put in a tube containing 0.2 mL trisodium citrate (9 parts blood to 1 part anticoagulant). Platelet poor plasma (PPP) was collected by centrifugation at 3000 × g for performance of PT, aPTT. These tests were done immediately after separation of plasma.
- 1ml of blood was collected in serum gel tube and centrifuged within 2 hours of collection for CRP.
- The remaining PPP was kept at -70° C for further analysis using the ELISA kit.

Statistical methods

Data were analyzed using IBM SPSS (version 24) statistical software package under Windows XP operating system for IBM compatible PC.

A- Descriptive statistics:

- Qualitative data were described in the form of number and percentage.
- Quantitative data were described in the form of mean ± standard deviation (SD), range and median.

B- Analytical statistics:

- Chi-Square test (X2): was used for comparison of qualitative data.
- Student's t-test: was used for comparison of quantitative data.
- Sensitivity (true positive rate): How good the test is at detecting disease.
- Specificity (true negative rate): How good the test is at identifying normal.
- Diagnostic accuracy (DA): Cases correctly classified.
- A receiver operating characteristic (ROC) curve: used to illustrate the diagnostic properties of a test on a numerical scale.
- Regression analysis: used to sort the markers according to their importance in discrimination between different studied groups of patients.
- P value: < 0.05 is considered significant.
- P value: <0.01 is considered highly significant.

- P value: ≥ 0.05 is considered insignificant.

The study was approved by the Ethics Board of Ain Shams University.

RESULTS

Eighty persons in this study were divided in to two groups: patient group (50 patients positive for DVT by duplex ultrasound) and control group (30 healthy volunteers).

I - Data of patient group

A- Site of DVT

DVT present in 50 patients. Most of patient (49 patient,99%) develop DVT in lower limb, while one patient was in upper limb (1%). This patient was on intravenous drug abuse.

B- Risk factors (Table 1)

As for risk factors, 3 (6%) patients were obese, 7 (14%) patients were smokers, 9 (18%) patients were recently bedridden for > 3 days and 5 (10 %) patients used oral contraceptive pills.

C- Wells score

All patients were greater than 3 with probability of deep venous thrombosis. The Wells score considers 1 point each for: active cancer, paralysis, paresis, recent plaster immobilization of lower limb, recently bedridden for >3 days or major surgery in past 4 weeks; localized tenderness along distribution of deep venous system; entire leg swollen; calf swelling >3 cm compared to asymptomatic leg; pitting edema and collateral superficial veins. A Wells score of 0 or 1 is associated with a low probability of venous thrombosis, a score of 2 with an intermediate probability of DVT and scores greater than 3 with a high probability of venous thrombosis [(5)].

II- Descriptive data of the studied groups (Tables 2&3):

In group I (50 patients positive for DVT by duplex ultrasound), the age ranged from 20 to 77 years, with a mean age of 46.20 ± 11.78 years. They were 41 (82 %) males and 9 (18 %) females, with a male to female ratio of 4.6:1.

In group II (30 healthy volunteers), the age ranged from 20 to 66 years, with a mean age of 44.9 ± 12.25 years. They were 23 (76.7%) males and 7 (23.3%) females, with a male to female ratio of 3.3:1.

III– Laboratory data

A- Hematological data of the studied groups (Tables 2&3):

In group I, WBC count ranged from 3.7 to 17.8 $\times 10^9$/L (mean 8.24 ± 3.09), Hb levels from 6.9 to 16 g/dL (mean 12.08 ±2.33), platelets count from 105 to 535 $\times 10^3$ /ul (mean 272 ± 90.47) and MPV ranged from 8 to 10.2 fl (mean 8.80 ± 0.65). PT ranged from 11 to 15.6 seconds (mean 13.53 ± 1.32), INR from 1 to 1.49 (mean 1.21 ± 0.16) and aPTT from 25 to 38 seconds (mean 32.40 ±.46).

In group II, the WBC count ranged from 3.9 to 18 $\times 10^9$/L (mean 9.07 ± 3.68), Hb level from 8.5 to 16 g/dL (mean 12.44 ± 1.97), platelets count from 110 to 530 $\times 10^3$/ul (mean 241.03 ± 88.95) and MPV ranged from 7.3 to 9.8 fl (mean 8.77 ± 0.64).PT ranged from 11 to 16.4 seconds (mean 13.84 ± 1.43), INR from 1 to 1.6 (mean 1.26 ± 0.18) and aPTT from 28to35 seconds (mean 31.8± 2.4).

B- Chemical investigation of studied group (Tables 2&3):

In group I, the ALT ranged from 19 to 44 (IU/L) (mean 30.68 ± 7.18) and AST ranged from 19 to 48 (IU/L) (mean 32. 54 ± 7.58), Creatinine ranged from 0.6 to 1.1 (mg/l) (mean 0.83 ± 0.15) and Urea ranged from 14 to 54 (mg/l) (mean 23.40 ± 7.80).

In group II the ALT ranged from 18 to 44 (IU/L) (mean 33.03 ± 8.21) and AST ranged from 19 to 46 (IU/L) (mean 34. 43 ± 8.56), Creatinine ranged from 0.6 to 1.1 (mg/l) (mean 0.85 ± 0.14) and Urea ranged from 14 to 54 (mg/l) (mean 24.47 ± 8.01).

- ***Comparison between different studied groups as regards hematological data and chemistry data.***

Regarding the comparison between group I (patients with positive duplex) and controls, no statistically significant differences were detected between both studied groups as regards the hematological parameters or chemical parameters as shown in Table (3).

C- CRP in studied groups (Table 4):

In group I, CRP ranged from 6 to 96 mg/l with a mean of (17.62±15.31).

while in group II, CRP was < 6 mg/l.

D- Serum -P selectin in studied groups (Table 5):

In group I, s-P selectin ranged from 70.5 to 109.5 (ng/ml) (mean 93.06± 6.52).

In group II, s-P selectin ranged from 4 to 27.5 (ng/ml) (mean 14.18± 5.59).

- ***Comparison between different studied groups as regards the measured markers:***

There was a highly significant difference between patients (group I) and controls (group II) as regards CRP and P-selectin (p = 0.000) as shown in Table (6).

IV - Correlation between P selectin and other laboratory data of patient

None of the studied clinical, haematological and chemical parameters showed any statistically

significant correlation with p-selectin in patient group, as shown in Table (7).

V - Correlation between risk factors and P selectin in patient group

None of the studied risk factors significantly affect s-P selectin as shown in Table (8)

VI - Performance characteristic of P-selectin as diagnostic markers for DVT (Table 9):

Receiver operator characteristic (ROC) curves were done to establish cut off levels for the diagnosis of DVT, threshold values were determined that differentiated control group from patient group. As regards P-selectin, a value of 70.5 ng/ml expression level was determined that had 98% sensitivity, 100% specificity with a NPV of 96.8% and a PPV of 100%.

Table (1): Risk factors of patient group.

Parameters		No.	%
Smoking	Negative Positive	43 7	86.0% 14.0%
OCP s	Negative Positive	45 5	90.0% 10.0%
Recently bed ridden for > 3 days	Negative Positive	41 9	82.0% 18.0%
Obesity	Negative Positive	47 3	94.0% 6.0%

Table (2): Descriptive and laboratory data of the studied groups.

Parameters		Patient group NO.=50	Control group NO.=30
Age (years)	Mean±SD	44.90 ± 12.25	46.20 ± 11.78
Sex	Female Male	7 (23.3%) 23 (76.7%)	9 (18.0%) 9 (18.0%)
PT (sec)	Mean±SD	13.84 ± 1.43	13.53 ± 1.32
INR	Mean±SD	1.26 ± 0.18	1.21 ± 0.16
PTT (sec)	Mean±SD	31.80 ± 2.40	32.40 ± 3.46
RBC (10^{12}/L)	Mean±SD	4.46 ± 0.56	4.33 ± 0.70
HB (g/dl)	Mean±SD	12.44 ± 1.97	12.08 ± 2.33
WBC (10^9/L)	Mean±SD	9.07 ± 2.24	8.24 ± 2.59
PLT (10^9/L)	Mean±SD	241.03 ± 59.25	272.76 ± 67.47
MPV (fL)	Mean±SD	8.77 ± 0.64	8.80 ± 0.65
ALT (IU/L)	Mean±SD	33.03 ± 8.21	30.68 ± 7.18
AST (IU/L)	Mean±SD	34.43 ± 8.56	32.54 ± 7.58
creatinine (mg/dl)	Mean±SD	0.85 ± 0.14	0.83 ± 0.15
urea (mg/dl)	Mean±SD	24.47 ± 8.01	23.40 ± 7.80

Table (1): Comparison between group I and group II patients as regards clinical and haematological parameters.

Parameters		Patient	Control	Independent t-test	
		No.=50	No.=30	t/Z*	P-value
PT (sec)	Mean±SD	13.53 ± 1.32	13.84 ± 1.43	0.997	0.322
INR	Mean±SD	1.21 ± 0.16	1.26 ± 0.18	1.311	0.194
PTT (sec)	Mean±SD	32.40 ± 3.46	31.80 ± 2.40	-0.836	0.406
RBC (10^{12}/L)	Mean±SD	4.33 ± 0.70	4.46 ± 0.56	0.867	0.388
HB (g/dl)	Mean±SD	12.08 ± 2.33	12.44 ± 1.97	0.716	0.476
WBC (10^9/L)	Mean±SD	8.24 ± 2.59	9.07 ± 2.24	1.073	0.287
PLT (10^9/L)	Mean±SD	272.76 ± 67.47	241.03 ± 59.25	-1.528	0.131
MPV (fL)	Mean±SD	8.80 ± 0.65	8.77 ± 0.64	-0.152	0.880
ALT (IU/L)	Mean±SD	30.68 ± 7.18	33.03 ± 8.21	1.345	0.182
AST (IU/L)	Mean±SD	32.54 ± 7.58	34.43 ± 8.56	1.030	0.306
cretinin (mg/dl)	Mean±SD	0.83 ± 0.15	0.85 ± 0.14	0.758	0.451
urea (mg/dl)	Mean±SD	23.40 ± 7.80	24.47 ± 8.01	0.586	0.559

PT: prothrombin time; aPTT: activated partial thromboplastin time; INR: international normalized ratio; WBCs: white blood cells; Hb: hemoglobin; PLT: platelets

Table (2): CRP in studied groups.

Parameters	Patient group NO.=50	Control group NO.=30
CRP (mg/l)	Median(IQR) 12.00 (10 - 24)	All are negative < 6(mg/l)
	Range 6 – 96	

Table (3): s-P selectin in studied groups.

Parameters		Patient group NO.=50	Control group NO.=30
P. selectin (ng/ml)	Mean ±SD	93.06 ± 6.52	14.18 ± 3.49
	Range	70.5 – 109.5	4 – 27.5

Table (4): Comparison between different studied groups as regards the measured markers.

Parameters		Patient	Control	Independent t-test	
		No.=50	No.=30	t/Z*	P-value
CRP (mg/l)	Median(IQR)	12.00 (10 - 24)	All are negative < 6(mg/l)	-6.731*	0.001
	Range	6 – 96			
P.selectin (ng/ml)	Mean±SD	93.06 ± 6.52	14.18 ± 5.59	-55.136	0.001
	Range	70.5 – 109.5	4 – 27.5		

Table (5): Correlation between P selectin and other laboratory data of patient.

Parameters	P.selectin (ng/ml)	
	r	p-value
Age	0.113	0.434
PT (sec)	-0.217	0.129
INR	-0.217	0.13
PTT (sec)	0.14	0.333
RBC (10^{12}/L)	0.159	0.269
HB (g/dl)	0.105	0.468
WBC (10^{9}/L)	0.009	0.951
PLT (10^{9}/L)	0.119	0.412
MPV (fL)	0.008	0.957
ALT (IU/L)	0.213	0.137
AST (IU/L)	0.149	0.302
Creatinin (mg/dl)	0.211	0.14
Urea (mg/dl)	0.227	0.113
CRP (mg/l)	0.228	0.111

Table (6): Correlation between risk factors and P selectin in patient group.

Parameters		P.selectin (ng/ml)		Independent t-test	
		Mean ± SD	Range	t	p-value
Sex	Female	93.61 ± 7.21	85 – 109	0.277	0.783
	Male	92.94 ± 6.45	70.5 – 109.5		
Site of DVT	Left Lower limp	92.95 ± 6.75	70.5 – 109.5	0.091	0.913
	Left Upper limp	95.00 ± 0.00	95 – 95		
	Right Lower limp	94.17 ± 3.79	91.5 – 98.5		
Smoking	Negative	93.07 ± 6.30	70.5 – 109	0.026	0.979
	Positive	93.00 ± 8.34	85 – 109.5		
OCPs	Negative	92.94 ± 6.31	70.5 – 109.5	0.372	0.711
	Positive	94.10 ± 9.03	87 – 109		
Recently bed ridden for > 3 days	Negative	93.70 ± 6.99	70.5 – 109.5	1.487	0.143
	Positive	90.17 ± 2.26	86 – 92.5		
Obesity	Negative	92.98 ± 6.51	70.5 – 109.5	0.346	0.731
	Positive	94.33 ± 8.10	85 – 99.5		

Table (7): Performance of P-selectin as diagnostic markers for DVT

Parameters	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
P.selectin	> 70.5	1.000	98.00	100	100	96.8

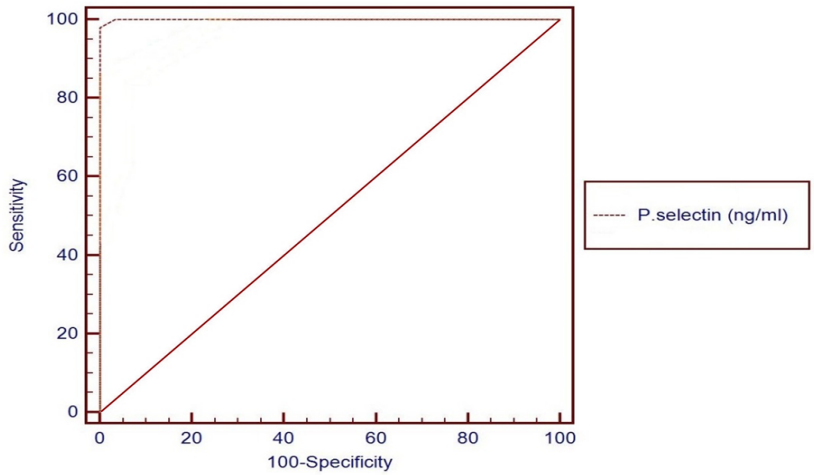

Figure (1): ROC curve showing the best cut-off values to differentiate control group and duplex positive group.

DISCUSSION

Venous thromboembolism (VTE) is a disease that includes both deep vein thrombosis (DVT) and pulmonary embolism (PE).

It is a common, lethal disorder that affects hospitalized and nonhospitalized patients. It is associated with increased long-term morbidity, disability, mortality, and high rates of recurrence. So accurate diagnosis of both is essential as delayed or missed diagnoses can result in death or longer term complications [(12)].

However, diagnosis cannot be based only on clinical presentation due to the lack of sensitivity and specificity of signs and symptoms [(13)]. Historically, the diagnosis of acute venous thromboembolism (DVT and PE) had depend primarily on imaging modalities including duplex ultrasound, helical CT scans, and venography, to establish the diagnosis of VTE. Currently, both imaging modalities and serological tests are utilized. These measured molecules are regarded as the biomarkers of DVT including D-Dimer, P-selectin, Factor VIII, thrombin generation, inflammatory cytokines, microparticles, fibrin monomer, D-Dimer, and IL-10 [(4)].

The use of plasma D-dimer testing was proved to be successful in excluding the presence of venous thrombosis, and it is the most commonly used clinical marker for VTE. A negative value of D-dimer may safely rule out both DVT and PE, with a high sensitivity up to 95%. However, due to its low specificity, D-dimer alone, even combined with clinical criteria, cannot be used to diagnose the DVT.

Its value also increases with recent surgery, trauma, or infection. Elevated levels are also seen with liver disease, pregnancy, and renal disease [(14)].

Some researchers reported that the adding P-selectin testing to the diagnostic algorithm has the potential to make the diagnosis of DVT more convenient and economical [(15)].

The aim of the current study was to determine the significance of P-selectin in DVT and whether it serves as a good clinical marker for DVT.

The current study was carried out on 80 individuals, who were divided into two groups: group I(50 newly diagnosed patient patients positive for DVT by duplex ultrasound) and group II (30 control subjects). All patients were evaluated as regards age, sex, body weight, medications, smoking history, personal or family history of DVT and history of recent surgery.

CRP by latex agglutination method and P-selectin assay by ELISA were measured in all patient. Similarly these marker were used also by **Barnes *et al.*[(4)]** and **Antonopoulos *et al.*[(1)]** as well as **Ramacciotti *et al.* [(16)].**

Comparison between studied groups as regards their demographic and different laboratory tests (hematological and chemical tests) revealed no significant difference between all groups. These results are in agreement with **Barnes *et al.* [(4)]** who studied three groups of individuals: 30 normal subjects, 22 patients positive for DVT on duplex ultrasound and 21 symptomatic patients, but negative for DVT on duplex ultrasound, and found no significant difference between three groups regarding their demographic data and laboratory tests.

In our study, we observed that serum p-selectin concentration did not correlate with the platelet count (age, height, weight, PT, INR, APTT, etc.) gender, and baseline medication or BMI This is in agreement with **Ay *et al.* [(6)]; Shi *et al.* [(15)]. Nagy *et al.* [(17)]** who found that no relation between p-selectin and any of the above parameters.

Regarding CRP level, it was significantly higher in patients group (Group I) than control group. In agreement with our results ***Gremmel et al.* [(18)]** studied two groups of individuals: 88 normal subjects and 44 patients with acute unilateral symptomatic DVT of the lower limb whose diagnosis of DVT was confirmed by color duplex sonography.

They recorded that CRP values at the time of diagnosis(using, hs-CRP with immunonephelometry) was significantly higher in patients than in healthy controls (1.16 mg/dL [range, 0.03-10.6 mg/dL] vs 0.15 mg/dL [range, 0.02-2.63 mg/dL].

Other studies also reported that CRP concentration was significantly higher in patient groups as opposed to control group [(16)]. The elevated levels of CRP in cases of DVT can be explained by inflammation which has been suggested as a risk factor for DVT: as patients with (DVT) manifest the four cardinal signs of inflammation heat, redness, pain, and swelling. It is known that the procoagulant thrombin is capable of stimulating multiple inflammatory pathways, and, also, inflammatory cytokines such as interleukin (IL)-6, IL-8, and monocyte chemotactic protein (MCP)-1 are capable of activating coagulation in VTE. Inflammatory cytokines may influence the expression of tissue factor, an initiator of the extrinsic pathway of coagulation,

thus providing a trigger that may lead to thrombotic disease [20].

However, in a prospective study, some researchers as **Tsai *et al.*** [21] measured the level of CRP in 19,237 adults with no baseline history of venous thromboembolism, cancer, or warfarin use. The endpoint was validated venous thromboembolism during follow-up (median, 7.8 years). A total of 159 venous thromboembolism events occurred and he found that there was no relationship between base line CRP levels and the subsequent development of VTE.

In addition, other studies evaluated that the use of plasma CRP level alone, does not appear to be useful to diagnose DVT [19].

Fox *et al.* [22] combined the data from the these studies and yielded a pooled weighted sensitivity of 77 % and specificity of 66%.Thus, plasma CRP level, used alone, does not appear to be useful to diagnose venous thrombosis.

Also **Ramacciotti *et al.*** [16] reported that CRP discriminated DVT positives from negatives but the p-value observed with CRP was lower than observed with sPsel. The standard deviation was also high (SD=±3.4) and even combining it with the Wells score or other biomarkers did not increase the sensitivity/specificity.

As regards P-selectin levels in the present study; a highly significant difference existed regarding its level, being significantly higher in group I (duplex positive patients) than the control group [1].

This finding can be explained by knowing that P-selectin translocate to the surface of activated platelets incorporated into a growing thrombus and supports the recruitment of circulating leucocytes. It also induces the expression of tissue factor on monocytes and mediates the binding of platelets to monocytes and neutrophils. As TF binds factor VII and activates factor IX and factor X, it is hypothesized that the P-selectin-induced TF synthesis on monocytes supports and maintains the local activation of blood coagulation in the hours following monocyte recruitment [26].

Using ROC curve, the diagnostic sensitivity and specificity of the different cut-off values for the studied marker in the current study were done. It was found that P-selectin cut-off point at 70.5 ng/ml showed 98% sensitivity and 100% specificity. This value was selected to differentiate between control subjects and patients with duplex documented DVT so that patients who have levels below these selected values can be safely regarded as DVT free and not given anticoagulant treatment.

In a study done by **Ramacciotti *et al.*** [16] who studied three groups of individuals: 30 normal subjects, 62 patients positive for DVT on duplex ultrasound and 116 symptomatic patients, but negative for DVT on duplex ultrasound, all from USA and found that ELISA assays which were used to evaluate circulating sPsel selectin was 28% sensitive and 96% specific at the cut-point 90 ng/ml.

Concerning P-selectin, **Ramacciotti *et al.*** [16], **Hou *et al.*** [20] **and Wang *et al.*** [25] used flow cytometry ,another method to measure P-selectin to establish the diagnosis of DVT, the cut-off was 17.8% expression level for P-selectin. While enzyme-linked immunosorbent assay was used to measure the level of P-selectin expression on platelets in our study, flow cytometry assay was used to evaluate circulating sP-selectin levels in the other studies. However, enzyme-linked immunosorbent assay more economic.

In other study ,**Ay *et al.*** [6] measured sP-selectin in 116 patients with confirmed recurrent VTE and invited all patients for a follow-up investigation after 3 months.They obtained blood samples for a 2nd sP-selectin measurement from 102 (88%) of the patients and 129 age- and sex-matched healthy individuals. Basal sP-selectin concentrations in acute occurrence were significantly higher in patients [mean (SD), 47.3 (15.0) ng/L] than in control individuals [36.8 (11.0) ng/L; P <0.001] .Thirty-four patients (29.3%) had sP-selectin concentrations greater than 55.1ng/L cutoff. And measured sP-selectin again 3 months after study entry in 102 of the 116 patients. sP-selectin concentrations at study entry [46.3 (15.8) ng/L] and after 3 months [47.8 (15.4) ng/L] were not significantly different. The mean difference between the 2 sP-selectin values was 2.2 (10.2) ng/L. The 1st and 2nd sP-selectin measurements were markedly correlated (r = 0.8). So Patients with venous thromboembolism (VTE) have demonstrated increased sP-selectin concentrations immediately after an acute event and at several months after VTE however increased sP-selectin concentrations are associated with VTE.

In other studies done by **Antonopoulos *et al.*** [1], patients with VTE measured sPsel concentrations immediately after an acute event and at several months after VTE they found that high levels of sPsel were recently associated with an increased risk for recurrence of DVT**.**

On the other hand, a decrease in plasma sPsel levels, after 7 days of therapeutic heparin therapy, have also been demonstrated. So it may be useful in follow up [16].

In follow-up study done by **Vandy *et al.*** [24], they established that a combination of Wells score ≥2 and a sP-sel >90 ng/mL could rule in the diagnosis of lower extremity DVT (LE-DVT) with a specificity of 95% and a positive predictive value of 100%. Conversely, a Wells score <2 and a sP-sel <60 ng/mL could rule out the diagnosis with a sensitivity of 99% and a negative predictive value of 96%.22.

High sPsel concentrations have been observed in other diseases including ischemic heart disease, atherosclerosis, acute ischemic stroke, DM, congestive heart failure peripheral artery disease, intermittent claudication, congestive cardiomyopathy, in addition to deep vein thrombosis [1].

Burger and Wagner [27] found a correlation between elevated platelet P-sel and the development of atherosclerotic lesions, the latter of which can lead to thrombosis.

In our study sP-selectin levels were not affected by age, gender, smoking, obesity, patients were recently bedridden for > 3 days and patients used oral contraceptive pills.

Similarly, **Hameed *et al.*** [28] found that no significant difference between sP-selectin and age, gender, smoking, arterial hypertension and hyperlipidaemia. However in other study they found that the level of sP-selectin in blood increases with deterioration of type 2 diabetes mellitus compensation and arterial Hypertension [29]. In our study patient diabetic and hypertension patient were excluded.

In other study related to body weight found that there is an association of higher P-selectin with risk of Chronic venous insufficiency (CVI) [30].

In a recent study the levels of platelet-derived microparticles and soluble P selectin can be used as novel early diagnostic marker of acute myocardial infarction [28].

Evaluation of sPsel at different time points during the evolution of VTE or during anticoagulation therapy will probably be a useful tool for surveillance and guidance of therapy for the patients with venous thromboembolic disease [1].

In conclusion, contrast venography is the most reliable way of diagnosing DVT, while it cannot be routinely used for screening purposes because of their uncomfortable invasion and complex procedures and compression ultrasound is also consider the current standard of care for the diagnosis of DVT. However, ultrasound is not always available, especially on the weekend, at night, or in smaller hospitals. In circumstances when ultrasound in not available, Therefore, biomarkers with high sensitivity and specificity are desirous of being identified for screening and early diagnosis of thromboembolism.

sP-sel takes these advantage of the biomarker associated with thrombosis and inflammation and differentiate patients who are free from DVT from others who are positive for DVT These biomarker in association with the clinical exam show early promise of making a laboratory diagnosis of DVT.

Furthermore it can be used in follow up the patient with DVT and will probably be a useful tool for surveillance and guidance of therapy for the patients with venous thromboembolic disease.

CONCLUSION

This study suggests the importance of P-selectin in assessing DVT. We conclude that the diagnostic cut-off levels of P-selectin in cases with DVT is 70.5 mg/L. that can safely differentiate patients who are free from DVT from others who are positive for DVT or those who will eventually develop thrombosis regardless their primary duplex ultrasound scanning results.

RECOMMENDATIONS

Serum P-selectin may be used as diagnostic biomarker in DVT; in addition to the newly estimated cut-off levels for these biomarker, further studies on larger number of cases are needed for more evaluation of these cut-off values and to establish whether they could be used to guide anticoagulation therapy when duplex ultrasound is unavailable.

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