

Role of P-Selectin In Patients with Slow Coronary Flow

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Abstract

Objective: To evaluate role of P-selectin as a marker of platelets activation in coronary slow flow patients.

Patients: A case control study, where seventy two patients underwent cardiac catheterization for suspected coronary artery disease. There were divided into patients group (primary coronary slow flow patients) and control group (normal coronary angiography).

Methods: All patients were subjected to history, physical examination and laboratory investigation included CBC serum glucose, lipid profile and immune phenotyping of platelets activation (p selectin CD 62p by flow cytometry).

Results: We have two groups: Group1 (patients group): Patients with primary coronary slow flow phenomenon=36 patients. Mean age of cases 49.33 ± 4.99 years with range of (34-55) years. In patients group there was 24 male and 12 female. Group II (control group): Patients with normal coronary angiography=36 patients. Mean age in control group 51.44 ± 3.36 years with range of (43-55) years.

Conclusion: The results of the present study revealed that there is very high statistically significant difference in P-selectin level between group 1 (primary coronary slow flow patients) and group 2 (normal coronary angiography patients) and there is statistically significant association between P-selectin and TIMI frame count in coronary slow flow.

Keywords: P-selectin; Slow coronary flow; Platelets; Hypercholesterolemia; Antioxidants

Introduction

The Coronary Slow Flow Phenomenon (CSFP) is an angiographic clinical entity, characterized by delayed distal vessel opacification in the absence of significant epicardial coronary stenosis [1].

The overall incidence of CSFP is 1% among patients who undergo coronary angiography, especially those presenting with acute coronary syndrome [2].

Several hypotheses of its mechanism including a form of early phase of atherosclerosis, micro vessel dysfunction, Hagen-poiseuille's equation, imbalance between vasoconstrictor and vasodilator factors, and platelet function disorder were proposed [3].

Platelets play a crucial role in the pathogenesis of atherosclerotic complications, contributing to thrombus formation after plaque rupture [4].

P-selectin is normally stored in the α -granules of platelets and the Weibel-Palade bodies of endothelial cells. Upon thrombogenic and inflammatory challenges, P-selectin is rapidly expressed, by exocytosis, on the cell surfaces of activated platelets and stimulated endothelial cell [5].

Subsequent to platelet activation, exposed P-selectin can bind with P-selectin glycoprotein ligand-1 (PSGL-1) of monocytes. These

aggregates can further enhance activation of monocyte, facilitating activation of inflammation and thrombosis. Platelet rolling and platelet endothelial adhesion are also mediated by P-selectin expression upon platelet activation [6].

P-selectin, expressed on the activated platelets, has been found to have a prominent role in stabilization of platelet aggregates and in size determination of platelet aggregates [7].

The patients with coronary slow flow have increased platelet activity compared to control subjects with normal coronary flow [8]. We therefore suggest that sP-selectin could be added as a possible biomarker to identify patients at cardiovascular risk.

Materials and Methods

Our study was a case control study, where all patients underwent cardiac catheterization for suspected coronary artery disease. The study was conducted in Zagazig – University Hospitals in clinical pathology department and catheterization laboratory from May 2017 till January 2018. We have two groups: Group1 (patients group): Patients with primary coronary slow flow phenomenon=36 patients. Mean age of cases 49.33 ± 4.99 years with range of (34-55) years. In patients group there was 24 male and 12 female with a male to female ratio 2:1. Group II (control group): Patients with normal coronary angiography=36 patients. Mean age in control group 51.44 ± 3.36 years with range of (43-55) years. In control group there was 20 male and 16 female.

Inclusion criteria

- Approval to share in the study
- Angiographically proven slow coronary flow
- Age from 18 to 55 years old.

Exclusion criteria

- Patient refusal.
- Local or systemic infection
- Previous history of infection
- Malignancy
- Known inflammatory or immunological disease
- Previous history of myocardial infraction
- Left ventricular dysfunction

All patients were subjected to

- Thorough history taking and physical examination stress on(history of smoking, chest pain ,DM,HPN)
- Laboratory Investigation

Sample collection

Blood samples were collected from patients and controls after an overnight fasting for 12 h. A total of 8 ml blood was collected from each subject.

Fresh EDTA blood (2 ml of blood transferred to K2 EDTA 3.6 mg vacutainer tube) was used for the assay of platelet activation, (2 ml of fresh blood samples were transferred to plain vacutainer tubes for assay of biochemical parameters and the rest of blood sample transferred to EDTA vacutainer for CBC examination.

Routine laboratory investigation

- Complete blood picture including ,Hb level, differential white blood cell and platelet count using Cobas Hitachi 8000 system.
- Serum glucose level and Complete lipid panel including (TC, LDL, HDL, Triglyceride) by Cobas 8000 system (Roche) after an overnight fasting.

Immunophenotyping of platelets activation(p selectin CD62p)

To minimize platelets activation during blood collection, we needed to use minimal stasis and large needle.

Blood was collected on k EDTA or sodium citrate vaccutianers. Fresh platelets rich plasma (PRP) samples (within less than 2hours) were collected and appropriately diluted Phosphate Buffer Saline (PBS) (1:1).

CD62p (p _selectin , GMP -1 leo) is a membrane glycoprotein in alpha granules of platelets. CD62p Monoclonal Antibody (MO Ab) purchased from NEW TEST company from Alexandria.

CD626 MOAb was labeled with Phycoerythrin(PE). Platelets activation was performed using FACS scan flowcytometry (Becton Dickinson, San Jose, CA, USA) based on the protocol described by Michelson (1999) with some modifications. Briefly 10 micron of MO Ab (cd62p) added to 100 micron of diluted sample, after gentle mixing sample were incubated for 15 minute in dark at room temperature.

Then washed twice with 2 ml PBS (5 min at 1500 rpm). The cell pellet was then resuspended in 500 micron of PBS . No fixative or lysing solution was used during the staining procedure.

Data acquisition was performed using CellQuest software (BD). Acquisition was done at Low flow speed and 10,000 events in the platelets region acquired. The forward and the side scatter (FSC/SSC) were set at logarithmic amplification and platelets were detected at the centered of the dot plot. For analysis, agate was set around platelets population in the FSC/SSC dot plot then a gated population was used to calculate the percentage of platelets positive for CD62p (Table 1).

	Angio free control group		PCSF group		χ ²	P value
	%	n	%	n		
Gender						
Male	20	55.6	24	66.7	0.935	0.334N.S
Female	16	44.4	12	33.3		
Age(years)						
X ± SD	51.44 ± 3.36		49.33 ± 4.99		1.109	0.271N.S
(range)	(43-55)		(34-55)			
Diabetes mellitus						
Absent	20	55.6	17	47.2	0.5	0.479 N.S
Present	18	44.4	19	52.8		
Hypertension						
Absent	16	44.4	20	55.6	0.889	0.346 N.S
Present	20	55.6	16	44.4		
Family history						
Absent	19	52.8	18	50	0.056	0.814N.S
Present	17	47.2	18	50		
Smoking						
Absent	21	58.3	18	50	0.503	0.478N.S
Present	15	41.7	18	50		

Table 1: Demographic data and chronic disease of studied group.

This table show that there is statistically non _significant differences between group 1 (PCSF) and group 2 (normal coronary angio) regarding age, gender, DM, HPN. Family history and smoking (Table 2).

	Angio free control group	PCSF group	t test	P value
Hemoglobin(g/dl)				
x ± SD	12.58 ± 1.53	13.39 ± 1.48	-2.301	0.024S
(range)	(10.1-15)	(10.8-16.5)		
WBCs(10³)				

x ± SD	6.91 ± 1.69	9.08 ± 3.69	-3.201	0.002*
median (range)	7.35 (4.5-104.6)	8.35 (4.8-25.7)		
Platelet(10³)				
x ± SD (*10³)	237.11 ± 37.39	227.42 ± 33.26	-0.571	0.57N.S
median (range) (*10³)	221 (166-292)	228 (144-289)		

Table 2: CBC findings among studied groups.

There is statistically significant difference between both groups regarding hemoglobin level (g/dl).

There is highly statistically significant difference between both groups regarding Total leucocytic count (10³). There is no statistically significant difference between both groups regard platelets count (10³) (Table 3).

	Angio free control group	PCSF group	t test	P value
Glucose(mg/dl)				
X ± SD	106.9 ± 10.45	111.84 ± 15.28	-1.6	0.114N.S
(range)	(83-120)	(82-137)		
T cholesterol				
	164.66 ± 34.14	186.31 ± 25.3	-3.058	0.003**
	(108.5-220)	(129.8-244)		
LDL c (mg/dl)				
X ± SD	117.42 ± 34.54	138.22 ± 24.12	-2.962	0.004**
(range)	(58.2-178)	(88.9-185)		
HDL c (mg/dl)				
X ± SD	45.55 ± 6.4	48.09 ± 10.46	-1.241	0.220N.S
(range)	(30.1-63)	(25.9-68.1)		
Triglycerides(mg/dl)				
X ± SD	137.42 ± 25.51	129.18 ± 41.81	1.01	0.317N.S
(range)	(95-180)	(72.1-222.1)		

Table 3: Fasting serum glucose (mg/dl) and (TC, LDL, HDL, Triglycerides, mg/dl) among studied groups.

This table shows there are statistically non-significant differences between both groups regarding fasting serum glucose.

There is a highly statistically significant difference between both groups regarding LDL. There is no statistically significant difference between both groups regarding HDL and Triglycerides.

There is high statistically significant differences between both regarding total cholesterol (Table 4).

	Angio free control group	PCSF group	t test	P value
P selectin				
x ± SD	8.08 ± 2.54	66.43 ± 20.69	-16.799	<0.001**
(range)	(2.38-12.91)	(36.6-87.23)		

Table 4: P-selectin value among studied groups.

This table shows that there is highly statistically significant difference between both groups regarding P selectin level (Table 5).

	Angio free control group	PCSF group	t test	P value
Gender				
Male	8.34 ± 1.82	61.22 ± 22.81	11.311-	<0.001**
Female	7.74 ± 3.27	76.84 ± 9.78	-23.515	<0.001**
Diabetes				
Absent	8.64 ± 2.38	64.28 ± 18.98	-11.997	<0.001**
Present	7.51 ± 2.65	68.35 ± 22.44	-11.733	<0.001**
Hypertension				
Absent	8.51 ± 2.5	66.29 ± 17.95	-14.247	<0.001**
Present	7.64 ± 2.59	66.59 ± 24.3	-9.567	<0.001**
Hypercholesterolemia				
Absent	7.85 ± 2.59	72.64 ± 12.02	-26.984	<0.001**
Present	9.92 ± 1.03	50.28 ± 29.36	-4.342	0.002**

Table 5: Relation between some risk factors and p selectin levels in study groups.

There are highly statistically significant differences between gender, presence of diabetes, hypertension and hypercholesterolemia and p selectin level (Table 6).

	(PCSF group)	
	r	p
Age (years)	0.012	0.943
Hemoglobin (g/dl)	-0.338	0.044*S
WBCs (x10³)	-0.137	0.427
Platelets (x10³)	-0.289	0.088
Glucose (mg/dl)	-0.073	0.672
LDL cholesterol (mg/dl)	-0.239	0.16
HDL cholesterol (mg/dl)	-0.31	0.066
Triglycerides (mg/dl)	0.041	0.812

Total cholesterol (mg/dl)	-0.356	0.033*S
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Table 6: Correlation between p selectin level and some laboratory parameters among (PCSF group)

Among cases, there are significant negative correlation between p selectin, haemoglobin level (g/dl) and Total cholesterol level(mg/dl) (Table 7).

	Angio free control group	PCSF group	t test	P value
LADc				
X ± SD	24.62 ± 3.06	55.72 ± 5.62	-27.61	<0.001**
CFX				
X ± SD	30.13 ± 32.3	49.71 ± 3.64	5.977-	<0.001**
RCA				
X ± SD	22.76 ± 2.95	52.61 ± 5.22	-29.31	<0.001**
Mean				
X ± SD	23.57 ± 2.73	52.66 ± 4.01	-33.42	<0.001**

Table 7: Comparison of TIMI frame count among study groups.

Cutoff	AUROC	Sensitivity	Specificity	PPV	NPV	+LR	-LR	accuracy	p
10.945	0.972	94.4	88.9	89.5	94.1	8.5	0.06	91.7	<0.001**

Table 9: Performance of P selectin in detection of slow coronary flow.

Discussion

The primary coronary slow flow phenomenon (PCSF) is an angiographic clinical entity, characterized by delayed distal vessel opacification in the absence of significant epicardial coronary stenosis [8].

PCSF has direct clinical implications, as it has been linked to clinical manifestations of myocardial ischemia, life-threatening arrhythmias, sudden cardiac death, and recurrent acute coronary syndromes [9].

Platelets play important role in the pathogenesis of atherosclerotic complications, contributing to thrombus formation after plaque rupture following platelet stimulation [10].

Platelet activation in patients with slow coronary flow remains to be largely unknown. Increased platelet activity in that patient group might be partially responsible for the development of acute coronary syndromes [11].

Platelets activation may be enhanced in the presence of micro vascular dysfunction or slow flow, including high shear stress and impairment of the endothelial barrier.

P-selectin is constitutively expressed in the a-granules of platelets and the Weibel-Palade bodies of endothelial cells, with a soluble form present in the plasma.

Following platelet stimulation, P-selectin becomes expressed on the platelet surface where it is rapidly shed. This shedding from platelets is thought to be the main source of the soluble form found in plasma

There is highly significant difference in (LADc, CFX, RCA and mean TIMI frame count) among study group (Table 8).

	Cases	
	r	P value
LADc TFC	0.482	0.003*S
CFX TFC	0.012	0.945N.S
RCA TFC	0.473	0.004*S
Mean TFC	0.451	0.006*S

Table 8: Correlation between p selectin level and TIMI frame count among cases.

This table shows that there is significant positive correlation between p selectin and LAD cTFC (left anterior descending artery TIMI frame count) and there is non-significant correlation between p selectin and CFX TFC (circumflex artery TIMI frame count). There is significant positive correlation between p selectin and RCA TFC (right coronary artery TIMI frame count). There is significant positive correlation between p selectin and LAD TFC (left anterior descending artery TIMI framecount (Table 9).

following thrombotic events and which may have its own physiological activity.

We tested the hypothesis that increased platelet activation may be present in patients with slow coronary flow (PCSF) and may contribute to the pathogenesis of primary coronary flow phenomenon (PCSF).

Our study was conducted in clinical pathology department and catheterization lab in Zagazig University hospitals to assess role of P SELECTIN in coronary slow flow. On 72 individual. Group I (Primary coronary slow flow); 36 case and Group II (Normal coronary angiography), 36 controls.

In this study regard CBC there is statistical significant difference in Hemoglobin level (g/dl) between both groups this was hand in hand with Singhal et al. who concluded that Hb increased the expression of P-selectin (secreted by α-granules upon platelet activation) in a concentration-dependent manner.

In this study the Total Leucocyte Count (TLC) is statistically significant different in PCSF than NCA, this is agree with Altunka et al. who found that there is significant difference between PCSF and agnio free controls.

As regaerd to platelets there was no statistical significance difference between both group. This was agree with Yilmaz et al. and disagree with Akboğa et al. where was higher TLC and platelets as there was positive correlation between them and CRP which indicate systemic inflammation but we exclude inflammation from our study.

Glucose in our study, there was no statistical significant difference between both groups, this was agree with Doğan et al. and disagree and disagree with Signori et al. due to increase incidences of metabolic syndrome in their study.

In our study, there was highly statistical significant difference between both groups regard LDL this was agree with Guo et al. who found high LDL (mg/dl) level in PCSF patients compared to controls. We found that there was no significant statistical difference between both group as regard HDL this was agree with Demir et al. this may genetic features of the both groups.

Regard Triglyceride there was no significant statistical difference between both group, this was agree with Doğan et al. and hand in hand with Yılmaz et al. he referred this due eating habits of the same country.

In our study cholestrol was significant statistically different between both group this was in agreement with George et al. and disagree with Doğan et al. this might due to different method enzymatic-colorimetric assay.

Regard P-selectin in our study there was highly statistical significance difference between both groups. This was agree with Celik et al. and disagree with Gurbel et al. they stated that the platelet expression of P-selectin is unrelated to the level found in plasma in patients with acute chest pain this might due to different determination method as they used ELISA.

Among cases there is highly statistical difference between gender and P SELECTIN.

In this study there is non-significant positive correlation between P selectin and age this was agree with George et al.

In our study among there was significant negative correlation between P SELECTIN and hemoglobin. This is agree with Singhal as they found that Hb increased the expression of P-selectin (secreted by α -granules upon platelet activation) in a concentration-dependent manner.

In our study there was non-significant negative correlation between p selectin and TLC (Total leucocyte count) this was agree with Ebeid et al. this was disagree with Aref et al. where they found positive significant correlation with P SELECTIN as control group were complete clinically free. In this study there was negative non-significant correlation between p selectin and platelet count.

In this study there was negative correlation between p selectin and LDL cholesterol this was in agreement with Chan et al. where they found incubation of HDL-C and oxidized LDL (ox-LDL) with platelets led to inhibition of the increased number of P-selectin receptors induced by ox-LDL. This was disagree with Ridker et al. as they measured them in patients with CAD and patients with metabolic syndrome.

We found that there was non-significant negative correlation between p selectin and HDL cholesterol. Guo who found non-significant correlation between P selectin and HDL.

In this study there was significant positive correlation between RCA TFC(right coronary artery TIMI frame count) and p selectin this was agree with Akboğa et al. and Pawelczyk et al. In this study there was significant positive correlation between mean TIMI frame count and p selectin this was in agreement with Demir et al. and Akboğa et al. there

was non-significant correlation between P selectin and CFX TFC (circumflex coronary artery TIMI frame count).

In this study when a ROC curve was generated to determine P selectin level in PCSF patients the area under the curve (AUC) was 0.972 The best cutoff value of P selectin in detection of slow coronary flow is ≥ 10.945 with sensitivity 94.4%, specificity 88.9, PPV 89.5, NPV 94.1, +LR 8.5, -LR 0.06. The accuracy of the test is 91.7 ($p < 0.001$). The current study demonstrated that sP-selectin levels of the patients with slow coronary flow were found to be significantly higher compared to those of the subjects with normal coronary flow. Besides, significant positive correlations were detected between mean TIMI frame counts and sP-selectin level.

Yetkin et al. have found that patients with myocardial infarction and normal coronary arteries have increased TIMI frame count, indicating the slow coronary flow; compared with subjects without myocardial infarction. They have suggested that SCFP might be the underlying cause of myocardial infarction in those patients.

The patients with coronary slow flow have increased platelet activity compared to control subjects with normal coronary flow. Although increased platelet activity may play a role in the pathogenesis of coronary slow flow.

Conclusion

In conclusion, the results of the present study revealed that there is very high statistically significant difference in P-selectin level between group 1(primary coronary slow flow patients) and group 2 (normal coronary angio patients) and there is statistically significant association between P-selectin and TIMI frame count in coronary slow flow. We therefore suggest that sP-selectin could be added as a possible biomarker to identify patients at cardiovascular risk.

Recommendations

We recommend performing a large scale population study on Egyptian patients to elucidate our results. Multiple and complex mechanisms are involved in the pathogenesis of PCSF, including; early phase of atherosclerosis, micro vessel dysfunction, Hagen-Poiseuille's equation model imbalance between vasoconstrictor and vasodilator factors, and platelet function disorder were proposed; therefore, much remains to be learned about these mechanisms and the functional contributions of P-selectin in each mechanism.

References

1. Arjmand N, Dehghani M (2016) Complete blood cell count components and coronary slow-flow phenomenon. *Ther Clin Risk Manag* 12: 1827-1829
2. Barbaux S, Poirier O, Pincet F, Hermand P, Tiret L, et al. (2010) The adhesion mediated by the P-selectin P-selectin glycoprotein ligand-1 (PSGL-1) couple is stronger for shorter PSGL-1 variants. *J Leukoc Biol* 87: 727-734.
3. Cleator J (2006) Differential regulation of endothelial exocytosis of P-selectin and von Willebrand factor by protease-activated receptors and cAMP. *Blood* 107: 2736-2744.
4. Heusch G (2016) Myocardial Ischemia. *Circulation Research*, 119: 194-196.
5. Hui H, Fuller K, Erber W, Linden M (2014) Measurement of monocyte-platelet aggregates by imaging flow cytometry. *Cytometry A* 87: 273-278.

6. Mutar M (2016) Risk Factors and Angiographic Findings in Patients with LBBB Who Underwent Coronary Angiography in Duhok Heart Center. *JMSR* 4: 14112-14117.
7. Wang X, Nie S (2011) The Coronary Slow Flow phenomenon: Characteristics, mechanisms and implications. *Cardiovasc Diagn Ther* 1: 37-43.
8. Baroopal A, Sanghvi S, Mathur R (2017) Clinical, demographic, risk factor and angiographic profile of coronary slow flow phenomenon: A single centre experience. *Indian Heart Journal* 69: S39-S40.
9. Wang X, Nie S (2011) The Coronary Slow Flow phenomenon: Characteristics, mechanisms and implications. *Cardiovasc Diagn Ther* 1: 37-43.
10. Badimon L, Vilahur G (2014) Thrombosis formation on atherosclerotic lesions and plaque rupture. *Journal of Internal Medicine* 276: 618-632.
11. Assinger A, Buchberger E, Laky M, Esfandeyari A, Brostjan C, et al. (2011) Periodontopathogens induce soluble P-selectin release by endothelial cells and platelets. *Thromb Res* 127: e20-26.