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Research Article

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Role of P-Selectin in Patients with Slow Coronary Flow

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ABSTRACT

Objective : To evaluate the 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. They 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 laboratort investigation including CBC serum glucose, lipid profile and immunophenotyping of platelets activation (p selectin CD 62p by flow cytometery). Results : There were two groups : Group1 (patients group) : Patients with primary coronary slow flow phenomenon = 36 patients. The mean age of cases was 49.33±4.99 years with the range of (34-55). In patients group, there were 24 males and 12 females. Group II (control group) : Patients with normal coronary angiography = 36 patients. The mean age in control group was 51.44±3.36 with the range of (43-55). Conclusion : The results of the present study revealed that there was a 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 was a statistically significant association between P-selectin and TIMI frame count in coronary slow flow.

Key words: *P-Selectin, Slow Coronary Flow, Platelets, Hypercholesterolemia, Antioxidants.*

INTRODUCTION

The coronary slow flow phenomenon (CSFP) is an angiographic clinical entity, which has been known by delayed distal vessel opacification in the absence of remarkable epicardial coronary stenosis [1].

The overall occurence of CSFP has been 1% among patients who undergo coronary angiography, especially those demonstrated 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 cells [5].

Subsequent to platelet activation, the exposed P-selectin can bind with P-selectin glycoprotein ligand-1 (PSGL-1) of monocytes. These aggregates can further enhance the activation of monocyte, facilitating the 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]. The researchers, therefore, have suggested that sP-selectin could be added as a possible biomarker to identify patients at cardiovascular risk.

MATERIALS AND METHODS

This 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. There were two groups : Group1 (patients' group) : Patients with primary coronary slow flow phenomenon = 36 patients. The mean age of cases was 49.33±4.99, with the range of (34-55). In patients' group, there were 24 males and 12 females with a male to female ratio of 2 :1. Group II (control group) : Patients with normal coronary angiography = 36 patients. The mean age in control group was 51.44±3.36 with the range of (43-55). In control group, there were 20 males and 16 females.

Inclusion criteria :

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

Exclusion criteria :

Patient's refusal
Local or systemic infection
Previous history of infection
Malignancy
Known inflammatory or immunological diseases
Previous history of myocardial infraction
Left ventricular dysfunction
All patients were subjected to :
Thorough history taking and physical examination stressing on (history of smoking, chest pain, DM, HPN)

Laboratory Investigation :

A- 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 samples were transferred to EDTA vacutainer for CBC examination).

B- Routine laboratory investigation

1. Complete blood picture including : Hb level, differential white blood cell, and platelet count using Cobas Hitachi 8000 system.
2. Serum glucose level and Complete lipid panel including : (TC, LDL, HDL, Triglyceride) by Cobas 8000 system (Roche) after an overnight fasting.
3. Immunophenotyping of platelets activation (p selectin CD62p).

To minimize platelets' activation during blood collection, it was needed to use minimal stasis and a 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 by phosphate buffer saline (PBS) (1 :1).

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

CD626 MOAb was labeled with phycoerythrin(PE). Platelets activation was performed using FACS scan flowcytometry (Becton Dickinsan, San Jose, CA, USA) based on the protocol described by Michelson (1999) with some modifications. Briefly, 10 micron of MO Ab (cd62p) was added to 100 micron of diluted sample. After gentle mixing, the samples were incubated for 15 minute in dark at room temperature, then washed twice with 2ml 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). The acquisition was done at Low flow speed and 10,000 events in the platelets' region were acquired. The forward and the side scatter (FSC/SSC) were set at

logarithmic amplification, and the platelets were detected at the centered of the dot plot. For analysis, a gate was set around platelets' population in the FSC/SSC dot plot, then, the gated population was used to calculate the percentage of platelets positive for CD62p.

RESULTS

Table 1 : Demographic data and chronic disease of studied groups

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

Table 1 shows that there were 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 : CBC findings among the studied groups

	Angio free control group	PCSF group	t test	P value
Hemoglobin (g/dl) x±SD (range)	12.58±1.53 (10.1-15)	13.39±1.48 (10.8-16.5)	-2.301	0.024S
WBCs (103) x±SD median (range)	6.91±1.69 7.35 (4.5-104.6)	9.08±3.69 8.35 (4.8-25.7)	-3.201	0.002**
Platelet (103) x±SD (*103) median (range) (*103)	237.11±37.39 221 (166-292)	227.42±33.26 228 (144-289)	-0.571	0.57N.S

There was a statistically significant difference between both groups regarding hemoglobin level (g/dl). There was a highly statistically significant difference between both groups regarding the total leucocytic count (103). There was no statistically significant difference between the both groups regarding platelets count (103).

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

	Angio free control group	PCSF group	t test	P value
Glucose(mg/dl) x±SD (range)	106.9±10.45 (83-120)	111.84±15.28 (82-137)	-1.6	0.114N.S
T cholesterol	164.66±34.14 (108.5-220)	186.31±25.3 (129.8-244)	-3.058	0.003**
LDL c (mg/dl) x±SD (range)	117.42±34.54 (58.2-178)	138.22±24.12 (88.9-185)	-2.962	0.004**
HDL c (mg/dl) x±SD (range)	45.55±6.4 (30.1-63)	48.09±10.46 (25.9-68.1)	-1.241	0.220N.S
Triglycerides(mg/dl) x±SD (range)	137.42±25.51 (95-180)	129.18±41.81 (72.1-222.1)	1.010	0.317N.S

Table 3 shows that there were statistically non-significant differences between both groups regarding fasting serum glucose. There was a high statistically significant difference between both groups regarding LDL. There was no statistically significant difference between the both groups regarding HDL and Triglycerides. There were high statistically significant differences between the both groups regarding their total cholesterol.

Table 4 : P-selectin value among the studied groups

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

Table 4 shows that there was a high statistically significant difference between the both groups regarding P selectin level.

Table 5 : The relationship between some risk factors and p selectin levels in the studied groups.

	Angio free control group	PCSF group	t test	P value
	x±SD	x±SD		
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**

As can be seen in Table 5, there were high statistically significant differences regarding gender, presence of diabetes, hypertension, hypercholesterolemia, and p selectin level.

Table 6 : The correlation between p selectin level and some laboratory parameters among (PCSF group).

	(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

Among the cases, there was a significant negative correlation between p selectin, haemoglobin level(g/dl) and Total cholesterol level(mg/dl) (See Table 6).

Table 7 : The comparison of TIMI frame count among the study groups.

	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**

According to Table 7., there were highly significant differences in (LADc, CFX, RCA and mean TIMI frame count) among the study groups.

Table 8 : The correlation between p selectin level and TIMI frame count among the cases.

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

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

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**

DISCUSSION

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

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

Platelets have played an 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 has remained to be largely unknown. Increased platelet activity in the patients' 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 [12].

P-selectin has been constitutively expressed in the a-granules of platelets and the Weibel–Palade bodies of endothelial cells, with a soluble form present in the plasma [13].

Following platelet stimulation, P-selectin has been expressed on the platelet surface where it has been rapidly shed. This shedding from platelets has been thought to be the main source of the soluble form found in plasma following thrombotic events, which may have its own physiological activity [14].

The researchers 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).

The study was conducted in a clinical pathology department and catheterization lab in Zagazig University hospitals to assess the role of P selectin in coronary slow flow on 72 individuals : Group I (Primary coronary slow flow) including 36 cases, and Group II (Normal coronary angiography) including 36 controls.

In the current study, regarding CBC, there was a statistical significant difference in Hemoglobin level (g/dl) between the both groups. This was hand in hand with [15] who concluded that Hb increased the expression of P-selectin (secreted by α-granules upon platelet activation) in a concentration-dependent manner.

In the present study, the Total leucocyte count (TLC) was significantly different in PCSF from NCA, this was in agreement with [16] who found that there was a significant difference between PCSF and agnio free controls.

Regarding platelets, there was no statistically significant difference between the both groups. This was in agreement with [17] and disagreement with [18] where was higher TLC and platelets, as there was a positive correlation between them, and CRP indicated a systemic inflammation, but the inflammation was excluded from this study.

Considering Glucose in this study, there was no statistical significant difference between the both groups, this was 0in disagreement with [19] due to the increase incidences of metabolic syndrome in their study.

In this study, there was a highly statistical significant difference between the both groups regarding LDL, which was in agreement with [20] who found high LDL (mg/dl) level in PCSF patients compared to the controls. It was found that there was no significant statistical difference between the both groups regarding HDL, which was in agreement with [21]. This might be because of the genetic features of the both groups.

Regarding Triglyceride, there was no significant statistical difference between the both groups, this was in agreement with [17]. This could be referred to the eating habits of the same country.

In the current study, T. cholestrol was significantly different between the both groups, which was in agreement with [22]. This might be due to the different methods of enzymatic-colorimetric assay.

Regarding P-selectin, in this study, there was a highly significant difference between the both groups. This was in agreement with [23], and in disagreement with [24], who stated that the platelet expression of P-selectin was unrelated to the level found in plasma in patients with acute chest pain which might be due to the different determination methods as used for ELISA.

Among the cases, there was a highly statistical difference between gender and P selectin.

In this study, there was a non-significant positive correlation between P selectin and age, which was in agreement with [22].

In this study, between the groups, there was a significant negative correlation between P Selectin and hemoglobin which was in agreement with [15] as they found that Hb increased the expression of P-selectin (secreted by α-granules upon platelet activation) in a concentration-dependent manner.

In the current study, there was a non-significant negative correlation between p selectin and TLC (Total leucocyte count) which was in agreement with [25], and in disagreement with [26] where they found a positive significant correlation with P selectin as control group was completely and clinicaly free. In this study, there was a negative non-significant correlation between p selectin and platelet count.

In this study, there was a negative correlation between p selectin and LDL cholesterol, which was in agreement with [26] where they found the incubation of HDL-C and oxidized LDL (ox-LDL) with platelets leading to the inhibition of the increased number of P-selectin receptors induced by ox-LDL, and was in disagreement with [27] as they measured them in patients with CAD and in patients with metabolic syndrome.

It was found that, there was a non-significant negative correlation between p selectin and HDL cholesterol. Which was in agreement with [20] who found non-significant correlation between P selectin and HDL.

In this study, there was a significant positive correlation between RCA TFC (right coronary artery TIMI frame count) and p selectin, which was in agreement with [18]. In this study, there was a significant positive correlation between mean TIMI frame count and p selectin, which was in agreement with [21] And [18]. There was a non-significant correlation between P selectin and CFX TFC (circumflex coronaty 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 was ≥10.945 with sensitivity of 94.4%, specificity of 88.9, PPV of 89.5, NPV of 94.1, +LR of 8.5, and -LR of 0.06. The accuracy of the test was 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, the significant positive correlations were detected between mean TIMI frame counts and sP-selectin level.

[28] have reported that the patients with myocardial infarction and normal coronary arteries have intensified TIMI frame count, demonstrating the slow coronary flow ; in comparison with the subjects without myocardial infarction. They have recommended that SCFP might be the underlying cause of myocardial infarction in those patients.

The patients with coronary slow flow have intensified platelet activity in comparison with control subjects with normal coronary flow. Although, the 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 was a 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 was a statistically significant association between P-selectin and TIMI frame count in coronary slow flow. The researchers, therefore suggested that sP-selectin could be added as a possible biomarker to identify the patients at the cardiovascular risk.

RECOMMENDATIONS

The researchers recommended performing a large scale population study on Egyptian patients to elucidate the results of the current study. Multiple and complex mechanisms can be involved in the pathogensis of PCSF, including ; early phase of atherosclerosis, micro vessel dysfunction, Hagen-Poiseuille's equation model, the imbalance between vasoconstrictor and vasodilator factors, and platelet function disorder. Therefore, much has remained to be learned about these mechanisms and the functional contributions of P-selectin in each mechanism.

REFERENCES

1. Arjmand, N. and Dehghani, M. (2016) : Complete blood cell count components and coronary slow-flow phenomenon. Therapeutics and Clinical Risk Management, 12(1) : 1827-1829
2. Barbaux, S., Poirier, O., Pincet, F., Hermand, P., Tiret, L. and Deterre, P. (2010) : The adhesion mediated by the P-selectin P-selectin glycoprotein ligand-1 (PSGL-1) couple is stronger for shorter PSGL-1 variants. Journal of Leukocyte Biology, 87(4) : 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(7) : 2736-2744.
4. Heusch, G. (2016) : Myocardial Ischemia. Circulation Research, 119(2) : 194-196.
5. Hui, H., Fuller, K., Erber, W. and Linden, M. (2014) : Measurement of monocyte-platelet aggregates by imaging flow cytometry. Cytometry Part A, 87(3) : 273-278.
6. Mutar, M. (2016) : Risk Factors and Angiographic Findings in Patients with LBBB Who Underwent Coronary Angiography in Duhok Heart Center. Journal of Medical Science And clinical Research, 04(11): 14112-14117.
7. Wang, X. and Nie, S. (2011) : The Coronary Slow Flow phenomenon : Characteristics, mechanisms and implications. Cardiovasc Diagn Ther., 1 : 37-43.
8. Baroopal, A., Sanghvi, S. and Mathur, R. (2017) : Clinical, demographic, risk factor and angiographic profile of coronary slow flow phenomenon : A single centre experience. Indian Heart Journal, 69 : pp. S39-S40.
9. Wang, X. and Nie, S. (2011) : The Coronary Slow Flow phenomenon : Characteristics, mechanisms and implications. Cardiovasc Diagn Ther., 1 : 37-43
10. Badimon, L. and Vilahur, G. (2014) : Thrombosis formation on atherosclerotic lesions and plaque rupture. Journal of Internal Medicine, 276(6) : 618-632
11. Assinger, A., Buchberger, E., Laky, M., Esfandeyari, A., Brostjan, C. and Volf, I. (2011) : Periodontopathogens induce soluble P-selectin release by endothelial cells and platelets. Thrombosis Research, 127(1).
12. Suner, A. and Cetin, M. (2016) : Is dipyridamole useful in improving left ventricular systolic and diastolic function in patients with coronary slow flow ? Echocardiography, 33(10) : 1472-1478
13. Barbaux, S., Poirier, O., Pincet, F., Hermand, P., Tiret, L. and Deterre, P. (2010) : The adhesion mediated by the P-selectin P-selectin glycoprotein ligand-1 (PSGL-1) couple is stronger for shorter PSGL-1 variants. Journal of Leukocyte Biology, 87(4) : 727-734.
14. Shigeta, A., Matsumoto, M., Tedder, T., Lowe, J., Miyasaka, M. and Hirata, T. (2008) : An L-selectin ligand distinct from P-selectin glycoprotein ligand-1 is expressed on endothelial cells and promotes neutrophil rolling in inflammation. Blood, 112 (13) : 4915-4923.
15. Singhal, A. Annarapu, A.K. Pandey, A. Chawla, Sh. Ojha, A. Gupta, A. 1Miguel A. Cruz, M.A. Seth, T. & Guchhait, T. (2015). Hemoglobin interaction with GP1bα induces platelet activation and apoptosis : a novel mechanism associated with intravascular hemolysis. Haematologica. 100(12) : 1526–1533.

16. Altunkas, F., Koç, F., Çelik, A., Kayihan, K., Karayakali, M. and Ceyhan, K. (2014) : Coronary slow flow phenomenon and neutrophil-to-lymphocyte ratio. Atherosclerosis, 235(2) : 215.
17. Yılmaz, M., Dağlı, M., Uku, Ö, Bilen, M., Korkmaz, H., Erdem, K. and Kurtoğlu, E. (2017) : Focusing on a complete blood cell parameter : mean platelet volume levels may be a predictor of coronary slow flow. Vascular Health and Risk Management, 13(1) : 255-261.
18. Akboğa, M.K., Canpolat, U., Balcı, K. G., Akyel, A., Şen, F., Yayla, Ç. and Aydoğdu, S. (2015) : OP-065 Increased Platelet-to-Lymphocyte Ratio is Related to Slow Coronary Flow. The American Journal of Cardiology, 115 : S28-S29.
19. Signori, L., Quadros, A., Sbruzzi, G., Dipp, T., Lopes, R. and Schaan, B. (2012) : Endothelial function in patients with slow coronary flow and normal coronary angiography. Clinics, 67(6) : 667-680.
20. Guo, L., Sun, G., Wang, G., Ning, W. and Zhao, K. (2014) : Soluble P-selectin promotes acute myocardial infarction onset but not severity. Molecular Medicine Reports, 11(3) : 2027-2033.
21. Demir, M., Cosar, S. and Melek, M. (2014) : Evaluation of plasma eosinophil count and mean platelet volume in patients with coronary slow flow. Clinics, 69 (5) : 323-326
22. George, K.M. Selvin, E. Pankow, J.S. Windham, B.G. Folsom, A.R. (2018). Respond to "Diabetes and Cardiovascular Disease". American Journal of Epidemiology,187(3), Pages 415–416.
23. Çelik, U. Togara, B. Turkez, H. Taspinar, N. (2014). In vitro cytotoxic, genotoxic, and oxidative effects of acyclic sesquiterpene farnesene. Turkish Journal of Biology, 38 : 253-259.
24. Gurbel, P.A, Kereiakes, D.J, Serebruany, V.L. (2000). Soluble P-selectin is not a surrogate marker for platelet P-selectin : evidence from a multicenter chest pain study group. J Thromb Thrombolysis.10(1) :15-22.
25. Ebeid, S., Sadek, N., El-Benhawy, S. and Mahmoud, G. (2014) : Plasma substance P and soluble P-selectin as biomarkers of β-thalassemia induced hypercoagulability. Alexandria Journal of Medicine, 50 (2) : 107-111.
26. Aref, S., Sakrana, M., Hafez, A. and Hamdy, M. (2005) : Soluble P-selectin levels in Diabetes mellitus patients with coronary artery disease. Hematology, 10(3) : 183-187
27. Ridker, P. M, Pare, G. Parker, A. (2008). Loci related to metabolic-syndrome pathways including LEPR, HNF1A, IL6R, and GCKR associate with plasma C-reactive protein : the Women's Genome Health Study. Am J Hum Genet. 82, pp. 1185–1192.
28. Yetkin, E. Turhan, H. Erbay, A.R. Aksoy, Y. Senen, K. (2005). Increased thrombolysis in myocardial infarction frame count in patients with myocardial infarction and normal coronary arteriogram : a possible link between slow coronary flow and myocardial infarction. Atherosclerosis, 181(1) :193-9.