*Journal of the Egyptian Society of Haematology & Research, Vol. 9, No. 1, March: 9-16, 2013*

# Role of Platelet Activation Markers CD62p and Annexine (V) in Hypertensive Patients With and Without Vascular Complications

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## ABSTRACT

***Background:*** Hypertension is a well known risk factor for cardiovascular and cerebrovascular events such as heart attack, renal failure and stroke. In addition, it is associated with earlier changes in target organ systems, such as LVH, proteinuria and carotid atherosclerosis, which are grouped under the term of "target organ damage" (TOD). There are many processes involved in the pathogenesis of TOD and some of these are endothelial dysfunction, platelet activation and increased thrombogenesis.

***Aim of the Work:*** This study aimed to study the Level of expression of P-selectin (CD62p) and Annexin V as platelets activation markers in hypertensive patients as compared to control group, and comparison between the levels of these markers in hypertensive patients with and without vascular complications aiming at early detection of vascular complication in hypertensive patients.

***Patients and Methods:*** *Our study included 50 patients divided into two groups:*

Group I: 20 patients suffering from hypertension without vascular complication. Group II: 30 patients suffering from hypertension with vascular complication which had renal, cardiac, cerebral and retinal affection. Also, 20 apparently healthy adults were chosen as control group. Patients were subjected to: Detailed history taking and clinical examination with emphasis on duration of disease and presence of complications. In the present study we investigated the levels of P-selectin (CD62p) and Annexin V expression on platelets by flow cytometry technique in patients with hypertension and control groups and compared the test characteristics of these markers. We also investigated the relation between P-selectin (CD62p) and Annexin V expression and various parameters;

***Results:*** A highly statistically significant increase in CD62p mean florescent intensity was detected in all hypertensive patients (without and with complication) compared with control group ($p<0.001$ and $p<0.0001$). A significant increase in CD62p mean florescent intensity in hypertensive patients with vascular complications compared to patients without vascular complications ($p<0.05$). A highly statistically significant increase in Annexin V mean florescent intensity was encountered in all hypertensive patients (without and with complication) compared with control group ($p<0.01$ and $p<0.001$), and in hypertensive patients with compared with patients without vascular complications ($p<0.05$). The results also showed significant correlation between (CD62p) mean florescent intensity and Annexin V mean florescent intensity in hypertensive patients with vascular complications groups only. CD62 and Annexin V did not correlate with creatinine, triglycerides, total cholesterol, serum LDL cholesterol or HDL cholesterol in any of the studied groups.

***Conclusion:*** The results of the present study support that activation of platelets in all hypertension patients is accompanied by high expression of P-selectin (CD62p) and Annexin V which is significantly associated with vascular complications. Hence, vascular complication may be predicted by an increase in the expression of these markers. The use of anti-platelet treatment in low-risk hypertensive patients and its effect on these markers needs to be investigated in long-term clinical outcome studies.

***Key Words:*** *Hypertension – CD62p – Annexine (V) – Vascular complication.*

## INTRODUCTION

Hypertension is a chronic medical condition, in which the blood pressure is chronically elevated. It is referred to as systemic arterial hypertension **[1]**. Hypertension is a well known risk factor for cardiovascular and cerebrovascular events such as heart attack, renal failure and stroke. In addition, it is associated with earlier changes in target organ systems, such as LVH, proteinuria and carotid atherosclerosis, which are grouped under the term of "target organ damage" (TOD). There are many processes involved in the pathogenesis of TOD and some of these are endothelial dysfunction, platelet activation and increased thrombogenesis **[2]**.

There is evidence that platelets and the endothelium, which both get activated in hypertension, have a crucial role in the increased thrombotic tendency seen in hypertension **[3]**. Abnormalities in platelet function may account for the pathogenesis and complications of thrombotic events associated with hypertension. Sev-

eral studies have reported that hypertensive patients show endothelial dysfunction and platelet hyperactivity and there is a positive linear relation between blood pressure and predisposition to platelet aggregation **[4]**.

Platelets play a key role in arterial thrombosis and acute vascular events. Activated platelets translocate and secrete P-selectin from their alpha granules. Once exposed on activated platelets, P-selectin on platelets interacts with leukocytes and then induces inflammatory signals to potentiate vascular injury **[5]**. P-selectin is an adhesion molecule located in the platelet alpha granules and Weibel-Palade body of endothelial cells. P-selectin mediates the rolling of blood cells on the surface of the endothelium and initiates the attachment of leukocytes circulating in the blood to platelets, endothelial cells, and other leukocytes at sites of tissue injury and inflammation **[6]**. In addition, P-selectin induces the expression of tissue factor on monocytes, thus initiating the blood coagulation cascade. It also mediates fibrin deposition in the growing thrombus, and induces superoxide anion production by neutrophils and monocytes. It also regulates production of platelet activating factor and phagocytosis by monocytes **[7]**. Soluble P-selectin (sP-selectin) is a biomarker for platelet/endothelial activation and is considered a risk factor for vascular disease **[8]**.

One of the most important signals accompanying platelet activation is the increase in intracellular calcium; annexins are highly conserved calcium-binding proteins, of which Annexin V is the major annexin in human platelets. The annexins are a family of proteins first described in 1990. All of the annexin proteins share the property of binding calcium and phospholipids. Annexin V has proven very useful as a marker for apoptosis and platelet activation **[9]**.

Circulating Annexin V can be released from the cells of the vascular wall (endothelial cells, smooth muscle cells) or from secretor cells of the spleen and liver. Once it is in the plasma, it binds to blood cells (platelets and erythrocytes) or to endothelial cells **[10]**.

Abnormalities in platelet function, endothelial function, and thrombotic markers have all been described in hypertension. Abnormal platelet aggregation, along with increased plasma markers of platelet activation, is also present in nonhypertension **[11]**.

The aim of this work was to study the Level of expression of P-selectin (CD62p) and Annexin V as platelets activation markers in hypertensive patients as compared to control group, and comparison between the levels of these markers in hypertensive patients with and without vascular complications aiming to evaluate its potential value for early detection of vascular complications in hypertensive patients.

## PATIENTS AND METHODS

***Subjects:***

This study was conducted on 50 patients with hypertension chosen from those attending Internal Medicine Department and Internal Medicine Clinic in AI-Zahraa University Hospital. They were 27 females (54%) and 23 males (46%) with an age ranging from 49 to 74 years old (Mean=60.24±7.25, Median=59). patient consider as hypertensive when either the systolic or the diastolic blood pressure value is >140/90 mmHg upon repeated sphygmomanometer measurements in the physician's office **[12]**. Twenty apparently healthy volunteers with matched age and sex were enrolled in the study as control group. They were 11 females (55%) and 9 males (45%). Cases with chronic inflammatory disease, infection, malignancy, Diabetes Mellitus, Smoking, and cardiac diseases due to causes other than hypertension were excluded.

*The studied cases were divided into two groups:*

Group I: Twenty patients suffering from hypertension without vascular complication. They were 13 females (65%) and 7 males (35%) with an age ranging from 49 to 60 years old (Mean=54±6, Median52).

These patients had no evidence of cardiac, retinal, cerebral, or renal affection.

Group II: Thirty patients suffering from hypertension with vascular complication who had renal, cardiac, cerebral and/or retinal affection. They were 14 females (46.7%) and 16 males (53.3%) with age ranged from 55-74 years old (Mean=67±7, Median62).

All patients and control were subjected to thorough history taking and complete clinical examination with emphasis on duration of disease, and presence of complications.

***Sample collection:***

Fasting sample: About 10ml of venous blood were withdrawn under sterile conditions in a

plastic syringe. The blood samples were divided into three parts:

1- Two ml added to (EDTA) tube for complete blood count (CBC) done using fully automated cell counter (Sysmix, Germany).

2- Five ml was left to clot and the serum was separated. Serum creatinine and lipid profile (triglyceride, HDL-Cholesterol, total cholesterol) were done using chemical auto analyzer (cobas 411) Germany and kits supplied by Roche Diagnostic Kits according to manufacture instructions.

3- The third part of blood sample (2.7ml) were added to 300µl 3.2% trisodium citrate and centrifuged immediately at low speed (100g) for 10 minutes using a cooling centrifuge for separation of platelet rich plasma (PRP) for isolation of fresh platelets for immunophenotyping.

***Flow Cytometric Measurement of CD62p and Annexin V:***

Platelet rich plasma was subsequently centrifuged at high speed (1000Xg) for 10 minutes with Phosphate buffered saline (PBS) to obtain washed platelets. Platelet pellet was washed twice and then re-suspended in an equal volume of 1% paraformaldehyde. Fifty µl of platelet suspension were stained with a combination of 10µl of Fluorescein isothiocyanate (FITC) conjugated anti CD62p (P-selectin); Kit supplied by BD Biosciences Pharmingen™ and 5µl Phycoerythrin (PE) conjugated anti Annexin V produced by BD Biosciences Pharmingen™. Iso type negative control (PE and FITC) was obtained from BD Biosciences Pharmingen™. The mixture was incubated in dark room for 30 minutes; they were resuspended in 200µl phosphate buffer saline (PBS) and centrifuged at 1300g for 5 minutes for analysis to be done. Data acquisition and analysis were performed on cell quest program of the FACSCAN, BEKTON DICKENSON flow cytometry. Gating on platelets, 10000 events were acquired and statistical analysis was done by cell quest software. Results were expressed as mean florescence intensity (Figs. 1,2).

***Statistical analysis:***

Data was analyzed by Microsoft office 2003 (excel) and (SPSS) computer program (version 16). Parametric data was expressed as Mean± SD. Spearman correlations coefficient was used to test the relationship between various variables. Significant difference was considered when *p*-value <0.05, *p*-value<0.01 is considered highly significant **[13]**.

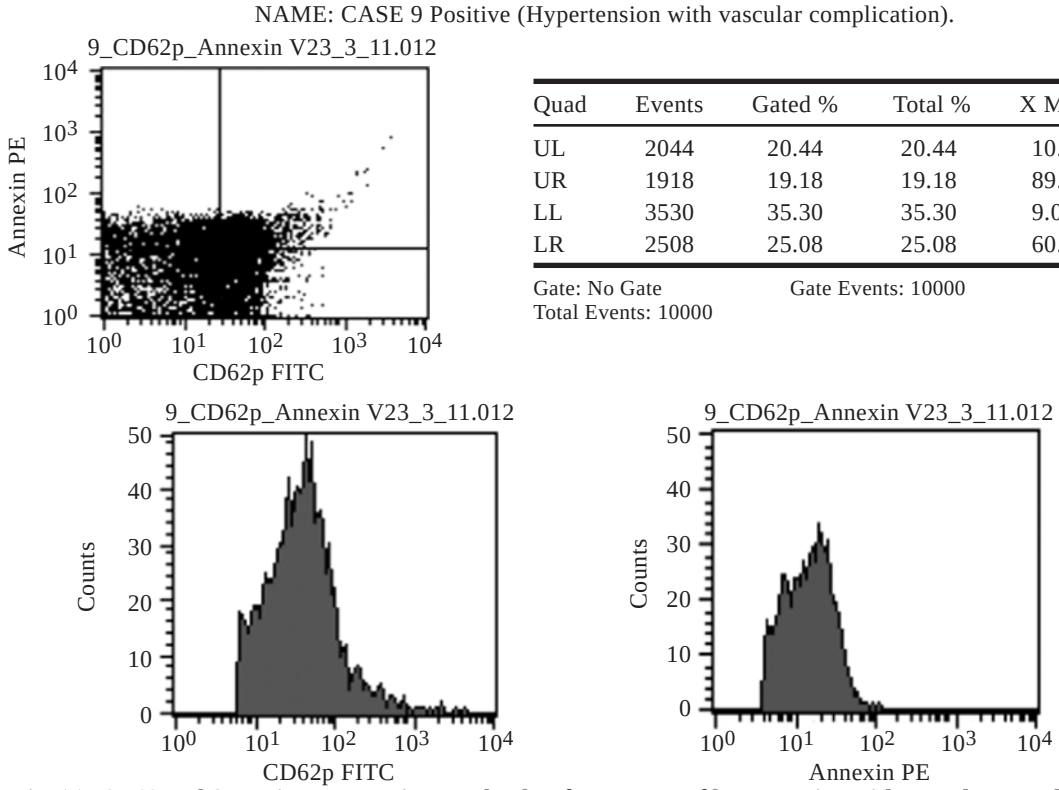


**Fig. (1): CD62 and Annexin V expression on platelets from a case of hypertension with vascular complications.**

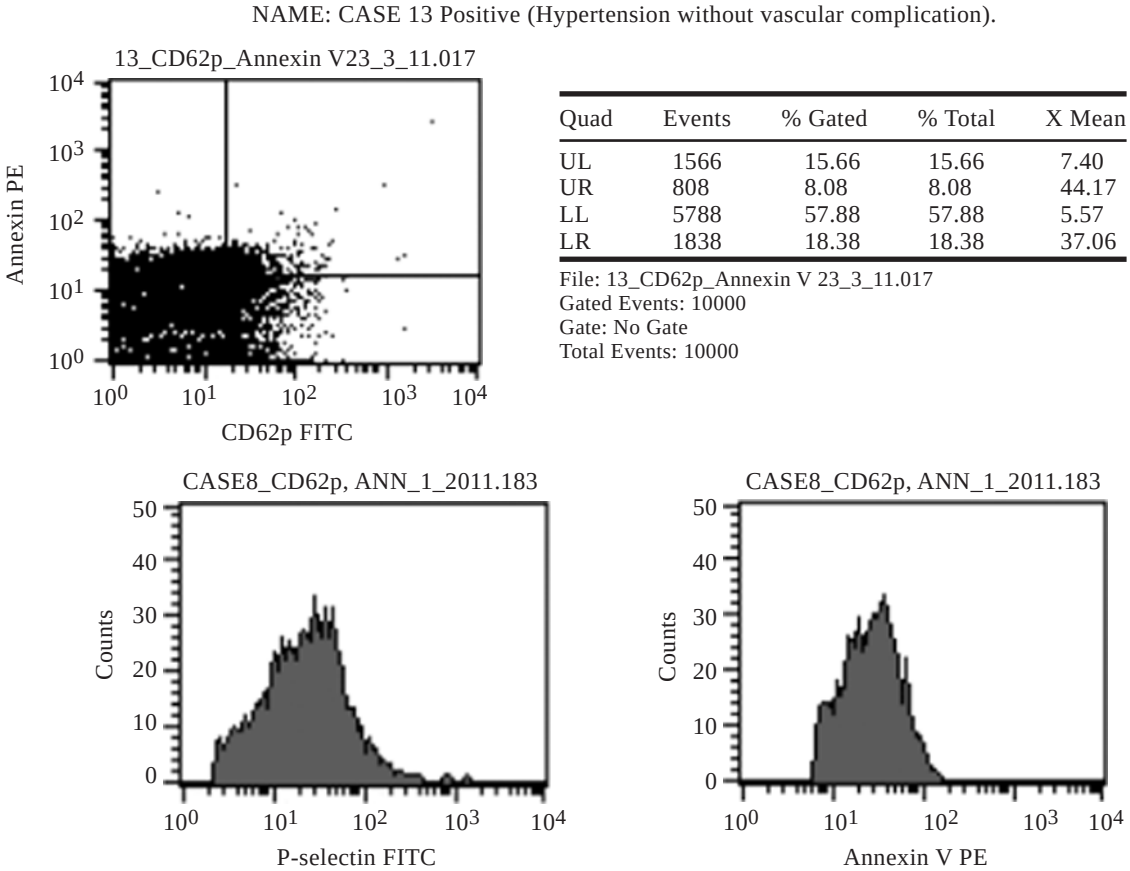


**Fig. (2): CD62 and Annexin V expression on platelets from a case of hypertension without vascular complications.**

## RESULTS

The results of serum creatinine and lipid profile are presented in Table (1). The values of the different parameters in hypertensive cases without vascular complications were comparable to control group ($p>0.05$). HDL cholesterol values were comparable in all groups ($p>0.05$). Serum creatinine, triglycerides, total cholesterol and LDL cholesterol was significantly higher in hypertensive patients with vascular complications compared to control group $p<0.0001$ and compared to hypertensive cases without vascular complication $p<0.001$.

CD62 and Annexin V expression on the platelets of the different groups are presented in Table (2). The mean florescent intensity of both markers was found to be significantly higher in hypertensive cases as compared to control as well as in hypertensive patients with vascular complications as compared to those without vascular complication.

A significant increase in CD62p mean florescent intensity was detected in all hypertensive patients (without and with complication) compared with control group ($p<0.001$ and $p<0.0001$). A significant increase in CD62p mean florescent intensity in hypertensive patients with vascular complications compared to patients without vascular complications ($p<0.05$). A highly statistically significant increase in Annexin V mean florescent intensity was encountered in all hypertensive patients (without and with complication) compared with control group ($p<0.01$ and $p<0.001$), and in hypertensive patients with vascular complication compared with patients without vascular complications ($p<0.05$).

Figures (3,4) represents Study of Correlation coefficient "*r*" between CD62p and Annexin V mean florescent intensity, Patients without vascular complication showed no correlation "*r*" 0.185, $p>0.05$*, Correlation was encountered in hypertensive patients with vascular complication "*r*" 0.385, $p<0.05$*. No correlation was encountered between CD62 or Annexin V and the other parameters in the studied groups.

Table (1): Creatinine and lipid profile in hypertensive patients with and without vascular complications and control group.

| Parameter | Hypertensive cases: Vascular complication | | Control | $p_1$ | $p_2$ | $p_3$ |
|---|---|---|---|---|---|---|
| | Absent | Present | | | | |
| No. | 20 | 30 | 20 | | | |
| Creatinine (mg/dl) | 0.87±0.28* | 4.65±4.66 | 0.79±0.27 | >0.05 | <0.0001 | <0.001 |
| Triglycerides (mg/dl) | 115.37±32.55 | 172.37±95.73 | 109.1±33.45 | >0.05 | <0.0001 | <0.001 |
| Total cholesterol (mg/dl) | 107.63±30.04 | 199.73±44.70 | 90.70±27.11 | >0.05 | <0.0001 | <0.001 |
| LDL cholesterol (mg/dl) | 52.66±21.12 | 143.30±54.55 | 47.40±20.53 | >0.05 | <0.0001 | <0.001 |
| HDL cholesterol (mg/dl) | 31.42±8.38 | 28.09±5.81 | 31.30±9.76 | >0.05 | >0.05 | >0.05 |

$p_1$: Hypertensive patients without vascular complications vs. control group.
$p_2$: Hypertensive patients with vascular complications vs. control group.
$p_3$: Hypertensive patients with vascular complications vs. hypertensive patients without vascular complications.

Table (2): P-selectin (CD62p) and Annexin V mean fluorescent intensity expression on platelets of hypertensive patients with and without vascular complications and control group.

| Study group | No. | CD6p | *p*-value | Annexin V | *p*-value |
|---|---|---|---|---|---|
| Hypertensive patients without vascular complications. | 20 | 17.98±0.91* | $p_1$<0.001 | 15.44±0.78* | $p_1$<0.01 |
| Hypertensive patients with vascular complications. | 30 | 24.06±0.93 | $p_2$<0.0001 | 19.34±1.01 | $p_2$<0.001 |
| Control group. | 20 | 6.29±1.01 | $P3$<0.05 | 7.47±0.95 | $P3$<0.05 |

*Mean florescent intensity±SD.
$p_1$: Hypertensive patients without vascular complications vs. control group.
$p_2$: Hypertensive patients with vascular complications vs. control group.
$p_3$: Hypertensive patients with vs. Hypertensive patients without vascular complications.

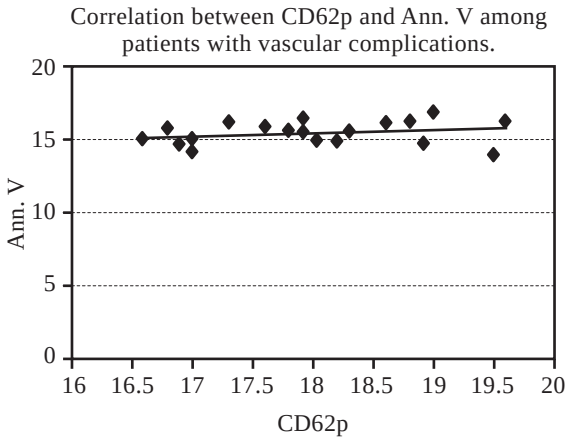


Fig. (3): Correlation coefficient "*r*" 0.185, $p$>0.05* between CD62p and Annexin V in hypertensive patients without vascular complications.

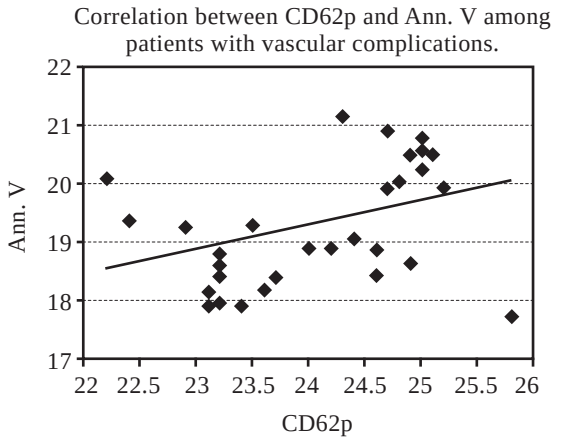


Fig. (4): Correlation coefficient "*r*" 0.385, $p$<0.05 between CD62p and Annexin V in hypertensive patients with vascular complications groups.

## DISCUSSION

In hypertension, the delicate balance between the vasodilators and the vasoconstrictors is upset, leading to changes that then take place in the vascular beds, There is also increasing evidence that platelets and the endothelium, both get activated in hypertension, The main complications of hypertension (that is, myocardial infarction and stroke) are paradoxically thrombotic in nature rather than hemorrhagic- "The thrombotic paradox of hypertension". Certainly, increasing clinical and laboratory evidence suggests that hypertension per se may

confer a prothrombotic or hypercoagulable state, with abnormalities of coagulation, platelets, and the endothelium **[14]**.

Platelets are activated by a large number of agonists that are released in the circulation during some pathologic conditions (e.g. hypertension and diabetes mellitus). Platelet activation and aggregation is involved in the development of hypertension in different ways. Indeed, upon activation, platelets change shape, release a number of autocrine factors that stimulate their adhesion to endothelial cells and the formation of blood clot. P-selectin and Annexin V have been proposed as useful biomarkers in various pathologic states in which platelets and/or endothelial cells are activated **[15]**. Hyperactive platelets may lead to capillary microembolisation secondary to the formation of microaggregates **[16]**.

This study was conducted to compare the level of expression of P-selectin (CD62p) and Annexin V as platelets activation markers in hypertensive patients and control group, and to compare between the level of expression of these markers in hypertensive patients with and without vascular complication aiming to early detection of vascular complication in hypertensive patients.

In the current study, the mean value of CD62p expression were higher in hypertensive Patients without vascular complications compared with control group. There was highly significant increase in CD62p mean florescent intensity in hypertensive patients with vascular complications compared to control group. These findings were in agreement with several studies **[17,18]**. Stumpf et al., **[19]** found that Patients with mild arterial hypertension (AH) showed significantly enhanced expression of platelet P-selectin and concluded that platelets seem to play a significant role in mediating inflammation in AH, which could lead to target organ injury.

In the current study, the mean value of CD62p expression was higher in hypertensive patients with compared to patients without vascular complications. This finding is in agreement with that of Yang et al., **[20]** who reported that the expression of CD62p in essential hypertension group was higher than the control group, and the level of the expression of CD62p in essential hypertension Grade II & III groups was obviously higher than that in essential hypertension Grade I group. Preston et al., **[21]** also reported that platelet CD62 demonstrated a strong and graded association with both systolic and diastolic BP; that association persisted in the presence of multiple concomitant risk factors. They reported, as well, that platelet activation and platelet CD62 expression increase in a BP-dependent manner and this relationship persists at extreme levels of BP. They concluded that Platelet activation and platelet CD62 may participate in the accelerated target organ injury observed in high risk patients with severe hypertension.

In the current study, the mean values of Annexin V mean fluorescent intensity expression was higher in hypertensive patients with or without vascular complications compared with control group. These findings are in agreement with previous study **[17]**. Also Sinning et al., **[22]** reported that Annexin V is increased in patients with cardiovascular risk factors and impaired coronary endothelial function and that this elevation is associated with many cardiovascular risk factors, such as hypertension, obesity, hyperlipoproteinaemia, and diabetes.

In the current study, the mean value of Annexin V mean florescent intensity expression was higher in hypertensive patients with compared to patients without vascular complications. This finding is in agreement with that reported by Huang et al., **[23]** who reported that hypertensive patients with microalbuminuria or macroalbuminuria had significantly increased Annexin V which may contribute to atherosclerotic disease progression and enhanced cardiovascular risk in hypertensive patients with nephropathy.

Chen et al., **[24]** reported increased levels of Annexin V, endothelial MPs (EMP) and platelet-derived MPs (PMPs), in diabetics with or without hypertension. Ravassa et al., **[25]** reported that myocardial Annexin V upregulation is associated with hypertensive heart disease (HHD) and impairment of systolic function in hypertensive patients; this association being independent of apoptosis. They concluded that Plasma Annexin V can be a marker of myocardial Annexin V up regulation and systolic dysfunction in patients with HHD.

Our study showed that there was no significant difference in serum triglycerides, total

cholesterol, LDL cholesterol or HDL cholesterol in hypertensive patients without vascular complications compared to control group, but that there was significant increase in serum level of triglycerides, total cholesterol and LDL cholesterol in hypertensive cases with vascular complications compared to control group, These results are in agreement with Alexandru et al., **[26]** who reported that hypertension is associated with hypercholesterolemia (cholesterol and triglyceride) that induces major changes in morphology and signaling mechanisms operating in blood platelets that enhanced platelet activation and aggregation in cardiovascular disease.

Our work showed that there was no significant correlation between CD62p, Annexin V and any of the other parameters (triglycerides level, total cholesterol, HDL cholesterol or LDL cholesterol) in all studied groups This is against the results obtained by Pawelczyk et al., **[27]** who observed a significantly higher CD62p expression and percentage of CD62p-positive resting and thrombin-activated platelets in the hyperlipidemia as compared to the normolipidemia group. This contradictory result may be due to the small number of cases in our study.

In this study there was significant correlations between CD62p mean florescent intensity and Annexin V mean florescent intensity in hypertensive patients with vascular complication but no correlation found in hypertensive patients without vascular complication. The present findings suggested that enhanced Platelet activation and platelet CD62 and Annexin V may participate in the accelerated target organ injury observed in high risk patients with uncontrolled hypertension.

Most clinical events associated with hypertension have a thrombotic component, and there have been several reports on platelet activation in hypertensive patients **[28,29]**. Our results for CD62p, and Annexin V support the findings in these reports.

*Conclusion:*

The results of the present study support that activation of platelets in all hypertension patients is accompanied by high expression of P-selectin (CD62p) and Annexin V which may induce hypercoagulability in hypertensive patients and significantly associated with vascular complications and hence, vascular complication may be predicted by an increase in the expression of these markers. The use of anti-platelet treatment in low-risk hypertensive patients and its effect on these markers needs to be investigated in long-term clinical outcome studies.

## REFERENCES


1- Carretero OA, Oparil S. “Essential hypertension” Part I: Definition and etiology. Circulation. 2000; 101 (3): 329-35.

2- Nadar SK, Tayebjee MH, Messerli F, Lip GY. Target organ damage in hypertension: Pathophysiology and implications for drug therapy. Curr Pharm Des. 2006; 12: 1581-1592.

3- Wong TY, McIntosh R. Hypertensive retinopathy signs as risk indicators of cardiovascular morbidity and mortality. Br Med Bull. 2005; 73-74:57-70.

4- El Haouari MA, Rosado JA. Platelet function in hypertension. Blood Cells, Mol Dis. 2009; 42: 38-43.

5- Cha JK, Jeong MH, Kim EK, Lim YJ, Ha BR, Kim SH. Surface expression of P-selectin on platelets is related with clinical worsening in acute ischemic stroke. J Korean Med Sci. 2002; 17 (6): 811-6.

6- Polek A, Sobiczewski W, Matowicka-Karna J. P-selectin and its role in some diseases. Postepy Hig Med Dosw (Online). 2009, 63: 465-70.

7- Gkaliagkousi E, Passacquale G, Douma S, Zamboulis C, Ferro A. Platelet activation in essential hypertension: implications for antiplatelet treatment. Am J Hypertens. 2010; 23 (3): 229-236.

8- Kisucka J, Chauhan AK, Zhao BQ. Elevated levels of soluble P-selectin in mice alter blood-brain barrier function, exacerbate stroke, and promote atherosclerosis. Blood. 2009; 113 (23): 6015-6022.

9- Tzima E, Walker JH. Platelet Annexin V: The ins and outs. Platelets. 2000, 11 (5): 245-51.

10- Cederholm A, Frostegård J. Annexin A5 as a novel player in prevention of atherothrombosis in SLE and in the general population. Ann NY Acad Sci. 2007; 1108: 96-103.

11- Minuz P, Patrignani P, Gaino S. Determinants of platelet activation in human essential hypertension. Hypertension. 2004; 43: 64-70.

12- Chobanian AV, Bakris GL, Black HR, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The JNC 7 report JAMA. 2003; 2892560- 2572.

13- Sokal RR, James RF. Biometry; the principle and practice of statistics in Biological Research. 2nd ed. New York: WH. Freeman. 1995.

14- Lip GY. Hypertension, platelets, and the endothelium: The ‘‘thrombotic paradox’’ of hypertension (or ‘‘Birmingham paradox’’) revisited. Hypertension. 2003; 41: 199-200.

15- Nomura S, Kanazawa S, Fukuhara S. Effects of efonidipine on platelet and monocyte activation markers in hypertensive patients with and without type 2 diabetes mellitus. J Hum Hypertens. 2002; 16 (8): 539-47.

16- Packham MA, Mustard JF. The role of platelets in the development and complications of atherosclerosis. Semin Hematol. 1986; 23: 8-19.

17- Hoffbrand AV, Moss PAH, Pettit JE. Platelets, blood coagulation and haemostasis. In Hoffbrand AV, Moss PAH and Pettit JE (editors): Essential Haematology, 5th edition, Blackwell publishing, Oxford. 2006; 22: 264.

18- Nomura S, Shouzu A, Omoto S, Nishikawa M, Fukuhara S, Iwasaka T. Losartan and Simvastatin Inhibit Platelet Activation in Hypertensive Patients. J Thromb Thrombolysis. 2004; 18 (3): 177-85.

19- Stumpf C, John S, Jukic J. Enhanced levels of platelet P-selectin and circulating cytokines in young patients with mild arterial hypertension. J Hypertens. 2005; 995-1000.

20- Yang H, Lang S, Zhai Z, Li L, Kahr WH, Chen P, Brkić J, Spring CM. Fibrinogen is required for maintenance of platelet intracellular and cell-surface P-selectin expression. Blood. 2009; 114 (2): 425-36.

21- Preston RA, Coffey JO, Materson BJ, Ledford M, Alonso AB. Elevated platelet P-selectin expression and platelet activation in high risk patients with uncontrolled severe hypertension. Atherosclerosis. 2007; 192 (1): 148-54.

22- Sinning JM, Losch J, Walenta K, Böhm M, Nickenig G. Circulating CD31+/Annexin V+microparticles correlate with cardiovascular outcomes. Eur Heart J. 2010; 3 2 (16): 2034-2041.

23- Huang PH, Huang SS, Chen YH, Lin CP, Chiang KH. Increased circulating CD31+/Annexin V+apoptotic microparticles and decreased circulating endothelial progenitor cell levels in hypertensive patients with microalbuminuria. J Hypertens. 2010; 28 (8): 1655-1665.

24- Chen Y, Feng B, Li X, Ni Y, Luo Y. Plasma endothelial microparticles and their correlation with the presence of hypertension and arterial stiffness in patients with type2 diabetes. J Clin Hypertens. 2012; 14 (7): 455-60.

25- Ravassa S, González A, López B, Beaumont J, Querejeta R, Larman M, Díez J. Up regulation of myocardial Annexin A5 in hypertensive heart disease: Association with systolic dysfunction. Eur Heart J. 2007; 28 (22): 2785-91.

26- Alexandru N, Popov D, Dragan E, Andrei E, Georgescu A. Platelet activation in hypertension associated with hypercholesterolemia: Effects of irbesartan. J Thromb Haemost Jan. 2011; 9 (1): 173-84.

27- Pawelczyk M, Baj Z, Chmielewski H, Kaczorowska B, Klimek A. The influence of hyperlipidemia on platelet activity markers in patients after ischemic stroke. Cerebrovasc Dis. 2009; 27 (2): 131-7.

28- Levy PJ, Yunis C, Owen J, Brosnihan KB. Inhibition of platelet aggregability by losartan in essential hypertension. Am J Cardiol. 2000; 86: 1188-1192.

29- Tomoda F, Takata M, Kagitani S, Kinuno H, Yasumoto K, Tomita S, Inoue H. Different platelet aggregability during mental stress in two stages of essential hypertension. Am J Hypertens. 1999; 12: 1063-1070.