Original Article-Pathology

A Study of Platelet Indices in Patients with Metabolic Syndrome and Type 2 Diabetes Mellitus

Mahadeo Mane[1], E Prabhakar Reddy[2], T.Mohanalakshmi[3]

[1]Professor of Pathology, D.Y. Patil Medical College, Kolhapur, Maharashtra, [2]Professor of Biochemistry and Central Laboratory Head, Sri Lakshmi Naryana Institute of Medical Sciences, Puducherry, Affiliated to BIHER, [3]Associate Professor of Microbiology, Sri Lakshmi Naryana Institute of Medical Sciences, Puducherry, Affiliated to BIHER

Abstract

Altered platelets have been reported in patients with diabetes mellitus and MS has been considered as a 'prothrombotic state' with enhanced platelet reactivity. They have been associated with increased risk of vascular complications in these patients. Platelet indices correlate with functional status of platelets and is an emerging risk factor of vascular complications in diabetes and MS. The study was undertaken to know the efficacy of platelet analysis in assessing the prognosis of diabetes mellitus and MS. Results of our study may lay a foundation for a discovery of new approaches for prevention and treatment of cardiovascular complications in MetS and DMT2 in early stages of metabolic disorders in patients. MetS is a proinflammatory and prothrombotic state, characterized by alteration of platelet indices. Plateletcrit was shown to be a significant biomarker along with other parameters, including waist circumference,SBP, and serum triglyceride levels. Platelet activation causes high risk of cardiovascular complications in MetS. T2 DM is associated with the changes in the intra cellular signaling systems regulating platelet functions.

Key words: ***P****latelets, Metabolic Syndrome, Type 2 Diabetes Mellitus, Nitric Oxide, Hematological parameters, Triglycerides.*

Introduction

Metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM) are the factors of cardiovascular risk. MetS comprises an array of pathogenetically interrelated metabolic and clinical abnormali- ties (insulin resistance, arterial hypertension, and dyslipidemia) and increases risk of atherosclerotic damage of blood vessels. Metabolic syndrome (MetS) is a combination of abdominal obesity, atherogenic dyslipidemia, elevated blood pressure (BP), and elevated plasma glucose [(1).] Platelets play a key role in the development of atherothrombosis,a major contributor of cardiovascular events [(1)]. The contribution of platelets to cardiovascular events has been noted for decades. Since then, there have been numerous studies underlying the importance of platelets in thrombotic complications [(2)]. The importance of platelets in cardiovascular disease, medicines aimed at inhibiting platelet activity have been demonstrated to be very effective at decreasing myocardial infarction, stroke. Metabolic syndrome, a precursor to diabetes, is an independent predictor of cardiovascular events [(3)].Although several measurements of platelet activity have emerged as potential contributors to atherothrombosis, many of these measurements are time-consuming,expensive, use a high sample volume, or require specialty training [(4-5)]. People with diabetes, exhibit increased platelet reactivity.Hyperglycemia contributes to greater platelet reactivity through direct effects and by promoting glycation of platelet proteins. Both insulin resistance and insulin deficiency increase

Corresponding Author:
Prof.Dr. E. Prabhakar Reddy
Professor of Biochemistry and Central Lab Head,
Sri Lakshmi Narayana Institute of Medical Sciences,
Puducherry, Affiliated to BIHER, Tamilnadu,India
Mobile No. +919159186879
Email id: drpebyreddy@yahoo.com

platelet reactivity. Insulin inhibits activation of platelets. Therefore, relative or absolute deficiency of insulin would increase platelet reactivity [6].

Recent studies on platelet volume indices in the spectrum of ischemic heart diseases show that all platelet indices—plateletcrit,mean platelet volume (MPV) and platelet distribution

width (PDW) were significantly raised in patients who had suffered from acute myocardial ischemia. A key component of vaso-occlusion is believed to be increased platelet activation and reactivity, and thus an increased platelet volume resulting in an elevated PDW. Also, higher concentrations of platelet microparticles have been detected in proinflammatory disorders such as sickle cell anemia. Studies on diabetes mellitus (DM), which too is considered to be a prothrombotic state with enhanced platelet reactivity, found a statistical rise in MPV and PDW, yet very few studies have been carried out regarding platelet indices for MetS patients.

MetS patients have been found to be at a greater risk for developing insulin resistance, visceral adiposity, atherogenic dyslipidemia, and endothelial dysfunction. Patients with insulin resistance tend to develop hypertriglyceridemia and are prone for developing atheromas in vascular lumen, leading to an increased incidence of coronary artery disease and stroke.8 Hypertriglyceridemia too leads to endothelial dysfunction that predisposes to the development of atherosclerotic depositions along vessel lumina. Visceral adiposity has been implicated in dysregulation of adiponectin levels, which too eventually causes vascular dysfunction. [7] Metabolic abnormalities, associated with insulin resistance syndrome, significantly affect functional activity of platelets. Activation of platelets can play an important role in progression of heart fail- ure due to a formation of the microthrombi in the myocardial microcirculation. Although several measurements of platelet activity have emerged as potential contributors to atherothrombosis,many of them are time consuming, expensive and use a high sample volume. Alternatively, MPV, PDW and P-LCR can be easily determined on routine automated hemograms available at low cost. Patients with larger platelets can easily be identified during routine haematological analysis and timely treatment could be undertaken.

Thus, in multiple recent studies, it has been shown that platelet indices are higher in patients suffering from DM, impaired fasting glucose, and dyslipidemia, as compared with MS. Thus, the aim of this study was to analyze the platelet indices in DM and MetS patients. To assess various platelet indices in MetS patients including plateletcrit, MPV, and PDW. To correlate platelet indices with other parameters of MetS, including waist circumference, BP, and lipid profile. The study also demonstrated an increase in the mean platelet diameter in T2DM patients. The effects of the pro inflammatory cytokines on functional activity of plate letsare studied. The present study compares the hematological parameters between patients with diabetes and MS.

Material and Methods

The study was carried between January to December 2019 in Central Laboratory of Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry, India. All the Samples were recruited and collected after informed consent and study was approved by Institutional Ethics Committee approval. Laboratory analysis The mean platelet volume (MPV), platelet count, and other blood cell indices were measured using a Study participants selected were 100 MetS patients aged between 25 and 60 years, attending the medicine OPD and/ or admitted to the medicine ward/intensive care unit (ICU).A DM, non-MetS participants were chosen. On assessment of waist circumference, BP was the parameter assessed next. Mean SBP, mean DBP On assessment by Student's unpaired *t*-test, Lipid profile of patients was then assessed. Fasting blood glucose levels of patients was the subsequent parameter taken into consideration. Platelet indices of patients, comprising plateletcrit , MPV, and PDW, were then taken into consideration. The first parameter taken into consideration was the patient's plateletcrit. When mean plateletcrit of the two categories was Compared.

Statistical Analysis

Statistical analyses were carried out using inferential statistics,including chi-square test and Student's *t*-test. Software used for the analysis was SPSS version 11.5.$p < 0.05$ being considered as significant.

Results

This study assessed morphologic, hematologic, and

biochemical parameters of 100 Metabolic syndrome and Diabetes patients.Criteria assessed to make the diagnosis of MetS were the NCEP-ATP III criteria.

Table 1. Correlation of Waist circumference of study population in Metabolic Syndrome and Diabetes Mellitus. **Table 2** Correlation of systolic blood pressure of study population in Metabolic Syndrome and Diabetes Mellitus. **Table 3** Correlation of serum triglyceride levels of study population in Metabolic Syndrome and Diabetes Mellitus. **Table 4:** Correlation of plateletcrit of study population in Metabolic Syndrome and Diabetes Mellitus

Table 1. Correlation of Waist circumference of study population in Metabolic Syndrome and Diabetes Mellitus

Category	N	Mean	Standard deviation	Standard error mean	t-Value	p-Value
Metabolic Syndrome	100	92.73	6.96	1.52	2.10	0.032
Diabetes Mellitus	100	83.78	5.18	1.40		

Table 2 Correlation of systolic blood pressure of study population in Metabolic Syndrome and Diabetes Mellitus

Category	N	Mean	Standard deviation	Standard error mean	t-Value	p-Value
Metabolic Syndrome	100	136.73	14.83	1.83	2.30	0.020*
Diabetes Mellitus	100	122.38	7.99	1.36		

Table 3 Correlation of serum triglyceride levels of study population in Metabolic Syndrome and Diabetes Mellitus

Category	N	Mean	Standard deviation	Standard error mean	t-Value	p-Value
Metabolic Syndrome	100	176.32	67.92	9.34	2.02	0.031*
Diabetes Mellitus	100	132.38	44.86	5.18		

Table 4: Correlation of plateletcrit of study population in Metabolic Syndrome and Diabetes Mellitus

Category	N	Mean	Standard deviation	Standard error mean	t-Value	p-Value
Metabolic Syndrome	100	0.23	0.07	0.012	2.42	0.016*
Diabetes Mellitus	100	0.20	0.02	0.002		

Discussion

On assessment by Student's unpaired *t*-test, no statistical significance was found in correlation of patients' age to their MetS status ($p < 0.05$). The findings we obtained in this study have been similar to those obtained by various other authors regarding work on both, MetS, and platelet indices in national and international literature. We obtained a significant, positive correlation of the MetS status of patients to their waist circumference, SBP, serum triglyceride levels, and plateletcrit, as confirmed using independent Student's unpaired *t*-tests. The role of platelet-activation pathways has also been widely studied in causation of complications in MetS. [8-9]. Studies have shown that platelet aggregation is controlled by the activity of PDI. This relationship between PDI and platelet hyper reactivity in MetS and its role in the causation of insulin resistance and nitric oxide dysfunction were studied by previous study [10]Increasing plateletcrit acts as a predisposing factor to development of cardiovascular complications in MetS patients, and also noted an increased risk of developing MetS among patients with increased leucocyte and erythrocyte counts [11-12]. Previous studies noted that elevated platelet indices correlated statistically with waist circumferences of patients and acted as a determinant of abdominal obesity, a major factor implicated in the development of MetS, with similar results shown by authors Barzin et al as well. [13-14]

Other platelet indices studied include platelet-to-lymphocyte ratio, which was found to be elevated in MetS patients, as well as shown be a marker for cardiovascular dysfunction in MetS patients [15-16] Further pathophysiology of platelet dysfunction and other biochemical abnormalities in MetS patients was studied in a study who documented sex-based differences in cardiovascular risk factors and predisposition to DM. [17].Increase in blood glucose level is one of the factors that change the erythrocyte morphology. The extent of change in shape of erythrocyte depends on the level of blood glucose level. All this affects the flow property of blood due to alteration and deformation [18]. The present study compares the hematological parameters between patients with diabetes and Metabolic Syndrome. Some studies performed a study on 170 diabetic patients to determine the relationship of glycemic control on hematological parameters in diabetes mellitus patients, reported that among hematological parameters MPV and PDW were significantly increased in diabetes patients [19]. Significant difference was obtained for RCDW, PDW, MPV, PLT and PCT between patients with good, poor and uncontrolled diabetes mellitus ($p<0.05$).Raised level of such indices can be utilized as the possible indicators for finding the risk of developing micro and macro vascular complication in diabetes patients [20] in present study total RBC, PVC and PLT were significantly high in patients with diabetes whereas RCDW was significantly high in Metabolic Syndrome patients.Some studies did not find any difference in haematological parameters except WBC count, all other parameters such as hemoglobin, platelets and haematocrit levels were similar in patients with and without diabetes which is in accordance with the present study findings [17].One study reported that platelet indices such as mean platelet volume and platelet distribution width were significantly

higher in diabetes patients, but in present study we found the contrary results [21].Patients with diabetes had higher level of total RBC, PCV and PLT whereas patients with hypertension and diabetes were having higher values of RCDW. Hematological parameters such as RCDW, PDW, MPV, PLT and PCT were found. Present study recommends the screening for hematological parameters and RFT in patients with diabetes mellitus.

Several publications provide evidence of putative inter relations between the MetS components and blood platelet count. One study showed that blood platelet number in the individ- uals presented with three and more MetS components is significantly higher than in the presence of only one or two components or without MetS components at all [22].Diabetes mellitus is not a single disease entity but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia. The chronic hyperglycemia and metabolic dysregulation may be associated with secondary damage in multiple organ systems, especially the kidneys, eyes and blood vessels [23].Platelets from patients with type 2 diabetes mellitus have increased reactivity and baseline activation which are likely to play a key role in development and sustainment of vascular complications [24]. Sustained hyperglycemia leads to a series of interrelated alterations that can cause evident endothelial dysfunction and vascular complications.

Human platelets are a nucleate discoid cells that circulate in the bloodstream and participate in hemostasis. In response to stimuli generated by the endothelium of blood vessels, platelets change shape, adhere to sub endothelial surfaces, secrete the contents of intracellular organelles, and aggregate to form a thrombus [24]. There is evidence that spontaneous platelet aggregation, estimated based on the sizes of the formed aggregates, was significantly higher in MetS patients compared with healthy volunteers. The curves of the mean aggregate sizes and light transmission characteristics suggested that the rates of collagen-induced aggregation of isolated platelets in MetS patients significantly exceeded the corresponding values in group of healthy volunteers [23-24]. The very elevated glycosylation of the platelet membrane proteins, rather than molar ratio of cholesterol and phospho- lipids, can decrease platelet membrane fluidity in diabetic patients[25]. Increased glycosylation of platelet proteins modulates cellular functions in diabetes. In particular, possible glycosylation of calmodulin that modulates activity of nitric oxide synthase (NOS) results in the decreased synthesis of nitric oxide (NO). Some data suggest that hyperglycemia contributes to platelet aggregation whereas normalization of glucose concentration attenuates this process [25]. High glucose concentrations hypothetically activate endothelial NOS in platelets via the osmotic mechanisms involving proteinkinase C-β isoform and intracellular calcium increase.

The molecule of NO is a universal regulator in the cardio- vascular, immune, and nervous systems of the organism. NO is synthesized both in endothelial, neural, smooth muscle cells, and in platelets. This molecule there by mediates auto regulation of platelet activity. NO is a neutral radical with unpaired electron. This molecule has the highest diffusion coefficient compared with other molecules (O_2 and CO_2) in the organism and free lypenetrates cellular membranes [25]. Activation of iNOS results in synthesis of high NO concentrations enough to stimulate T-cell-mediated immunity and exert cytotoxic effects. iNOS is identified in macrophages, neutrophils, keratinocytes, fibroblasts, chondrocytes, osteoklasts, neurons, astrocytes, various epithelial cells (respiratory, retinal, pigment, renal, tubular, and adeno carcinomatous), hepatocytes, pancreatic β-cells, endotheliocytes, endocardiocytes, and vascular smooth musclecells. The enzyme is activated by cytokines or bacterial antigens in inflammation as well as by ultraviolet, ozone, nicotinic acid, and hormones affecting cAMP synthesis(adrenalin, glucagon).This NOS isoform generates manifold NO amount compared with other forms of NOSs and does not require Ca2+ for this process. Our study demonstrated an increased basal NO production by monocytes isolated from MetS patients perhaps due to the iNOS activation. There is evidence of decrease in the insulin stimulated NO production by the endothelial and smooth muscle cells in the presence of insulin resistance [25]. India betes, platelet adhesion and spontaneous aggregation increase as well. Altered activity of NOS in platelets from patients with MetS can play the key role in onset of the platelet hyper activation and development of macro and micro angiopathies. Studies of NO production in the platelets showed decreased basal NO production in all groups of MetS patients compared with healthy donors. Moreover, the lowest rates of NO synthesis

wereobservedinpatientswithdecompensatedT2DM. There is reverse correlation between the NO mediated synthesis of cGMP in platelets and the levels of glucose and glycated hemoglobin in blood. Reduced basal formation of NO by the cells from MetS patients can because by various reasons. The presence of hyperglycemia due to glucose autoxidation contributes to the formation of superoxide an ion interacting with NO and mediating formation of the peroxinitrite attenuating NO content. There is evidence that platelets express functional Ca2+-calmodulin dependent constitutive NOS. Glycosylation of calmodulin can cause reduction of basal NO secretion.

Human platelet aggregation is also modulated by NO and prostacyclin (PGI2) released from the endothelium. Endothelial dysfunction, accompanying the insulin resistance syndrome, attenuates NO and PGI2 production in the endotheliocytes. Effects of NO and PGI2 on the platelets are mediated by the adenylate and guanylate cycases that synthesize cAMP and cGMP. The adenylate and guany- late cyclases are regulated by prostacyclin and NO, respectively. Formed cGMP inhibits phosphordiesterases metabolizing cAMP. Degradation of cAMP and cGMP is mediated by phosphodiesterases [(26)]. Abnormalities in cAMP/cGMP pathways can cause platelet hyper- activity. Platelets from patients with diabetes have decreased sensitivity to NO and PGI2. In diabetes mellitus, the number of PGI2 receptors is not decreased suggesting that the defect is downstream of the receptor. Previous studies [(26)] detected a decrease in G*i* protein in the platelet membranes fromT2 DM patients. Some studies demonstrated elevated activity of cGMP dependent phosphodiesterase eventually leading to decrease in sensitivity to NO. Homocysteine affects blood vessels through the active oxygen forms, decrease in endothelial NO production, and augmentation of smooth muscle cell proliferation. Homocysteine can decrease platelet NO production by several mechanisms. Striated that homocysteine causes atherogenic effects in diabetic patients due to decreased platelet NO production with following augmentation of platelet activity and aggregation. The effects of insulin on platelets in healthy individuals consist in the NO-mediated elevation of cGMP and cAMP, decrease in Ca2+ currents induced by Ca2+ mobilizing agents, decrease in agonist- induced aggregation, augmentation of platelet binding with anti aggregation prostanoids, and suppression of platelet binding with catecholamines leading to adrenalin induced platelet aggregation. In insulin resistant patients, calcium content in platelets rises in response to stimulation by insulin, leading thereby to platelet activation and aggregation. The study of individuals with abnormal carbohydrate metabolism demonstrated augmentation of platelet activity and aggregation in response to an increase in content of cGMP exert- ing anti-aggregation effect. The authors assume that elevated platelet aggregation in these patients causes cGMP increase by the negative feedback mechanism.

Conclusion

Platelet activation causes high risk of cardiovascular complications in MetS. T2 DM is associated with the changes in the intra cellular signaling systems regulating platelet functions. Due to the altered NOS expression and activity, platelets increase their prothrombogenic potential. The cGMP-mediated anti aggregational effects of insulin, involving NOS and NO-dependent mechanisms, become abnormal. The effects of aggregation triggering agonists are modulated by the proinflammatory factors. All these mechanisms of changes in platelets aggregation activity in MetS and DM T2 are caused by the metabolic disturbances including insulin resistance, hyperglycemia, and dyslipidemia.

Results of our study may lay a foundation for a discovery of new approaches for prevention and treatment of cardiovascular complications in MetS and DM T2 in early stages of metabolic disorders in patients. Patients with metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM) have high risk of micro circulation complications and micro angiopathies. Factors leading to platelet activation in MetS and T2 DM comprise insulin resistance, hyperglycemia,non-enzymaticgly cosylation,oxidativestress,andinflammation.Decrease in a number and sensitivity of the insulin receptors on platelets in T2DM cancause platelet hyperactivation. These data suggest a potential role of platelet activity measurement in subjects with diabetes. Thus, platelet volume indices MPV, PDW and P-LCR provides an important, simple, effortless and cost effective tool which can be useful in predicting an impending thrombotic state and vascular complications of diabetes. Plateletcrit was shown to be a significant biomarker along with

other parameters, including waist circumference, SBP, and serum triglyceride levels. Early detection using these markers can lead to an overall decline in morbidity and mortality due to MetS. MetS is a proinflammatory and prothrombotic state, characterized by alteration of platelet indices. Plateletcrit was shown to be a significant biomarker along with other parameters, including waist circumference, SBP, and serum triglyceride levels.

Conflict of Interest: Nil

Source of Funding: Self/Diagnostic kits are provided by institution as on complimentary basis for research.

Ethical Clearance No: No.IEC/C:135/2019

References

1. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R et al (2003) Expert committee on the diagnosis and classification of diabetes mellitus2. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003; 26:3160–3167.
2. Davì G, Patrono C. Platelet activation and atherothrombosis. N Engl J Med 2007; 357:2482–2494.
3. Gami AS, Witt BJ, Howard DE, et al.Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol 2007; 49:403–414.
4. Michelson AD. Methods for the measurement of platelet function. Am.J.Cardiol 2009; 103(Suppl.):20A–26A.
5. Nicholson NS, Panzer-Knodle SG, Haas NF, et al. Assessment of platelet function assays. Am Heart J 1998; 135(5 Pt 2 Su): S170–S178.
6. Keating FK, Sobel BE, Schneider DJ. Effects of increased concentrations of glucose on platelet reactivity in healthy subjects and in patients with and without diabetes. Am J Cardiol 2003; 92:1362–1365.
7. Stratmann B, Tschoepe D.Pathobiology and cell interactions of platelets in diabetes. Diab Vasc Dis Res 2005; 2:16–23.
8. van Rooy MJ, Pretorius E. Metabolic syndrome, platelet activation and the development of transient ischemic attack or thrombo embolic stroke. Thromb Res 2015; 135(3):434–442.
10. Gaspar RS, Trostchansky A, Paes AM. Potential role of protein disulfide isomerase in metabolic syndrome-derived platelet hyperactivity. Oxid Med Cell Longev 2016; 24(2)35-47.
11. Zhou P, Meng Z, Liu M, et al. The associations between leukocyte,erythrocyte or platelet, and metabolic syndrome in different genders of Chinese. Medicine (Baltimore) 2016; 95(44):e5189.
112. Zhao F, Yan Z, Meng Z, et al. Relationship between mean platelet volume and metabolic syndrome in Chinese patients. Sci Rep 2018; 8(1):14574.
13. Furman-Niedziejko A, Rostoff P, Rychlak R, et al. Relationship between abdominal obesity, platelet blood count and mean platelet volume in patients with metabolic syndrome. Folia Med Cracov 2014; 54(2):55–64.
14. Barzin M, Asghari G, Hosseinpanah F, Mirmiran P, Azizi F. The association of anthropometric indices in adolescence with the occurrence of the metabolic syndrome in early adulthood: Tehran Lipid and Glucose Study (TLGS). Pediatr Obes 2013; 8(3):170–177.
15. Akboga MK, Canpolat U, Yuksel M, et al. Platelet to lymphocyte ratio as a novel indicator of inflammation is correlated with the severity of metabolic syndrome: a single center large-scale study. Platelets 2016; 27(2):178–183.
16. Akboga MK, Canpolat U, Yayla C, et al. Association of platelet to lymphocyte ratio with inflammation and severity of coronary atherosclerosis in patients with stable coronary artery disease. Angiology 2016; 67(1):89–95.
17.. Asmah RH, Yeboah G, Asare-Anane H, Antwi-Baffour S, Archampong TN, Brown CA, *et al.* Relationship between oxidative stress and haematological indices in patients with diabetes in the Ghanaian population. Clinical Diabetes and Endocrinology. 2015; 1(7):1-5.
18. Singh M, Shin S. Changes in erythrocyte aggregation and deformability in diabetes mellitus. Indian Journal of Experimental Biology. 2009; 47(1):7-15.
19. Jabeen F, Rizvi HA, Aziz F, Wasti AZ. Hyperglycemic induced variations in Hematological Indices in Type 2 Diabetics. International Journal of Advanced Research. 2013; 1(8): 322-34.
20. Jabeen F, Rizvi HA, Subhan A. Effect of

hyperglycemia on superoxide dismutase defensesystem and erythrocyte indices in diabetic patients. Pak J Biochem Mol Biol. 2012; 45(2): 85-9.

21. Biadgo B, Melku M, Abebe SM, Abebe M. Hematological indices and their correlation with fasting blood glucose level and anthropometric measurements in type 2 diabetes mellitus patients in Gondar, Northwest Ethiopia. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2016; 9:91-9.
22. Kotani,K., Sakane,N.,Saiga, K.,Mu,H., and Kurozawa,Y..Clustered components of the metabolic syndrome and platelet counts in Japanese females. *Clin. Chem.Lab.Med*. 2007; 45,376–379.
23. Pickering TG, Hall JE, Appel LJ, et al. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. Circulation 2005; 111 (5):697–716.
24. Ryo M, Nakamura T, Kihara S, et al. Adiponectin as a biomarker of the metabolic syndrome. Circ J 2004; 68 (11):975–981.
25. Vinik, A.I and Erbas,T. Diabetic autonomic neuropathy. *Handb. Clin. Neurol*.2013; 117,279–294..
26. Smolenski, A. Novel roles of cAMP / cGMP-dependent signaling in platelets. *J. Thromb. Haemost*. 2012; 10,167–176.