MEAN PLATELET VOLUME AND PLATELET FUNCTION ANALYSIS IN ACROMEGALIC PATIENTS BEFORE AND AFTER TREATMENT

M. Demirpence¹, H.Y. Yasar^{1,*}, A. Colak², B. Akinci³, S. Yener³, B. Toprak², I. Karademirci²

Tepecik Research and Training Hospital, ¹Dept. of Endocrinology, ²Dept. of Biochemistry, Izmir, ³Dokuz Eylul University Hospital, Dept. of Endocrinology, Aydin, Turkey

Abstract

Objective. Mean platelet volume (MPV) and platelet function analysis have been studied before in acromegaly, but the effect of treatment on both parameters has not been evaluated. We aimed to investigate MPV and platelet function analysis in acromegalic patients after six-months of treatment.

Methods. Forty patients with active acromegaly and 36 healthy subjects were included in the study. Plasma glucose and lipids, fibrinogen, GH, IGF-1 levels, MPV and platelet function analysis were measured. All patients with acromegaly were re-evaluated six months after treatment.

Results. Fasting blood glucose (FBG), GH, IGF-1, fibrinogen levels and MPV values were significantly higher in acromegalic group compared with the control. Platelet function was enhanced significantly (pcol-ADP: 0.002, pcolepinephrine: 0.002). After 6 months of treatment FBG, serum GH, IGF-1, fibrinogen and MPV decreased and collagen/ADP- and collagen/epinephrine-closure times (CT) were increased. Acromegalic patients that were in remission with long-acting SSA after surgery had significantly higher fibrinogen levels and MPV and decreased collagen/epinephrine-CT with respect to the controls (pfibrinogen: 0.001, pMPV: 0.026, pcol-epinephrine: 0.037).

Conclusion. Acromegaly was associated with increased MPV and enhanced platelet activity. Although growth hormone hypersecretion was controlled by surgery and medical treatment, these parameters did not improve – indicating a still increased risk for cardiovascular events.

Key words: Acromegaly, platelet function analysis, mean platelet volume .

INTRODUCTION

Acromegaly is the result of excessive growth hormone hypersecretion. It is associated with increased cardiovascular risk, whose pathogenesis is not completely identified (1). Cardiovascular abnormalities include hypertension, left ventricular hypertrophy

and cardiomyopathy, characterized by diastolic dysfunction and arrhythmias (2). Other cardiovascular risk factors include abnormal glucose metabolism (hyperinsulinism, impaired glucose tolerance or overt diabetes) and lipid profile (low levels of high-density lipoprotein (HDL)-cholesterol, high levels of small dense low-density lipoprotein (LDL)-cholesterol, triglyceride (TG), Lipoprotein (Lp) (a) (1, 3).

Due to increased prevalence of atherosclerosis, the reported prevalence of coronary artery disease in acromegalic patients ranges from 3% to 37 % in different studies (1, 4). Besides, there are several studies about coagulation and fibrinolysis in acromegalic patients as possible risk factors for cardiovascular disease (1, 5-9). We previously evaluated coagulation parameters (protein C, protein S, fibrinogen) and platelet function analysis (performed with collagen/ADP and collagen-epinephrine) in patients with acromegaly. We observed increased tendency to coagulation and enhanced platelet activity.

In addition to platelet function analysis, changes in the size, density and reactivity of platelets may be involved in the natural history of vascular disease. MPV the accurate measure of platelet size is a marker of platelet function (10). Larger and functionally more reactive platelets increase the propensity to thrombosis (11). Elevated MPV values have been found in coronary heart disease (12, 13). In addition, increased platelet size has been reported in patients with vascular risk factors such as diabetes, hypertension, hypercholesterolemia, smoking, renal artery stenosis (14), polycystic ovary syndrome (11) and SCH (15, 16). There are four studies which investigated MPV value in patients with acromegaly (17-20), however, only Ucler *et al.* studied the MPV values in acromegalic patients both before and after treatment (surgery and/or medical treatment). But, to our knowledge there is no data about the effect of treatment on platelet function analysis in

*Correspondence to: Hamiyet Yılmaz Yasar MD, Tepecik Research and Training Hospital, Department of Endocrinology, Izmir, Turkey, E-mail: drhamiyetyilmaz@yahoo.com

acromegalic patients. Therefore, we aimed to evaluate MPV and platelet function analysis in patients with active acromegaly and the effect of treatment on these parameters.

PATIENTS AND STUDY DESIGN

The study was a single-center, prospective follow-up in patients with active acromegaly. Forty patients attending to our outpatient Endocrinology Clinic of Tepecik Research and Training Hospital with active acromegaly (age range, 33-71 years) were recruited as acromegaly group between August 2013 and November 2015. The diagnosis of acromegaly was previously established by the typical clinical signs and symptoms (acral enlargement, soft tissue overgrowth, hyperhidrosis), by a failure of GH levels to suppress below 1ng/mL during a standard (75 g) oral glucose tolerance test (OGTT) associated with increased IGF-1 values for age and sex. Pituitary imaging was obtained by magnetic resonance imaging (MRI) and 28 (72.5%) patients had macroadenomas and 12 (27.5%) had microadenomas. All patients were untreated at enrollment and had active acromegaly at the beginning of the study. Most of them, 38 patients, had undergone selective resection of pituitary adenomas by the transsphenoidal approach and 2 patients had been administered primary medical therapy as somatostatin receptor ligands (octreotide-LAR), since the patients had refused to undergo surgery. Also somatostatin receptor ligands (octreotide-LAR or lanreotide) were given to patients who had undergone surgery and had still elevated GH and IGF-1 levels. The patients who had hypopituitarism were taking adequate replacement treatment.

A total of 36 healthy persons (16 men, 20 women), mean age 47.3 ± 4.7 years, attending our Family Practice outpatient clinic just for check-up were included as the control group. None of the study participants in the control group were taking any medical treatments (estrogen, anti-hyperlipidemic therapy or treatment that affect hemostatic parameters) or had diseases (diabetes mellitus, dyslipidemia, thyroid dysfunction, atrial fibrillation, renal disease, liver cirrhosis) that might affect blood coagulation and fibrinolysis, and lipid profile.

Also, all participants were non-smokers or none of them had family history of clotting disorders and none of them were receiving any medication that might affect blood coagulation and fibrinolysis.

All patients with acromegaly were re-evaluated

by physical examination and laboratory assessment six months after treatment (surgery and/or medical treatment).

The study was approved by the medical ethics committee of the Tepecik Research and Training Hospital and all participants provided written informed consent.

Body mass index (BMI) was measured in all study subjects. BMI was calculated by the ratio between weight and height squared in kg/m². Blood pressure was measured with the person in a seated position after a 5 minute rest with an Omron M3 HEM-7131 electronic, auscultatory blood pressure reading machine. The first reading was discarded, and the mean of the next three consecutive readings was used.

After an overnight fast of 12 hours, venous blood was collected from the antecubital vein for the evaluation of the following biochemical parameters: plasma glucose and lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides), fibrinogen, MPV values, platelet function analysis performed with collagen/ADP and collagen-epinephrine and IGF-1 levels GH levels were assessed during 75 g OGTT suppression test. Blood for haemostatic assays was collected in 3.2% sodium citrate in a 9 to 1 blood to anticoagulant ratio and processed within 1 h of venipuncture. Also ABO blood group was analyzed in all study participants.

Laboratory Assessments

Glucose concentrations were measured by a hexokinase method with the Olympus AU-2700 analyzer. Triglycerides, total cholesterol and HDL-cholesterol were measured by an enzymatic method with Olympus AU-2700 analyzer using reagents from Olympus Diagnostics (GmbH, Hamburg, Germany). LDL-cholesterol was calculated by the Friedewald's equation method. Serum GH and IGF-1 levels were measured by a chemiluminescent immunometric assay (Immulin XPI, Siemens, Germany). The normal range for IGF-1 was age dependent (21-25 years; 116-358, 26-30 years; 117-329, 31-35 years; 115-307, 36-40 years; 109-284, 41-45 years; 101-267, 46-50 years; 94-252, 51-55 years; 87-238, 56-60 years; 81-225, 61-65 years; 75-212, 66-70 years; 69-200, 71-75 years; 64-188, 76-80 years; 59-177 ng/mL). Plasma fibrinogen levels (reference range, 200 - 400 mg/dL) were determined using ACL coagulation analyzer system (Instrumentation Laboratories, Lexington, MA). MPV was measured in a blood sample collected in citrate (1:4 v/v)1. A Cell-Dyn 3500 (Abbot) was used for

complete blood counts. Platelet count determination was performed by a hematology cell counter, Sysmex (Roche) utilizing flow cytometry method.

Platelet function was evaluated by the platelet function analyzer (PFA-100) (21) a US Food and Drug Administration–approved device (Dade Behring), was used for measuring platelet function. It aspirates blood *in vitro* from a blood specimen into disposable test cartridges through a microscopic aperture cut into a biological active membrane at the end of the capillary. The membranes of the cartridges are coated with collagen and adenosine diphosphate (ADP) or collagen and epinephrine inducing a platelet plug to form which closes the aperture. The time passed between the aspiration of the blood to the closure of the aperture and termination of the flow of blood is called closure time (CT). Normal collagen/epinephrine CT is 85–165 seconds and collagen/ADP CT 71–118 seconds (22).

Statistical Analysis

Results are expressed as means $\pm$ SD. The patient and the control group were compared by using Student-t test. The Chi-Square test was used for non-parametric variables. Basal and after-treatment values of the study group were compared by using paired samples t-test. MANOVA test and Bonferroni adjustment were used to compare the subgroups of acromegalic patients and the control group and two-

group comparisons were performed by Student-t test. $P < 0.05$ was considered statistically significant. Statistical analysis was performed with SPSS 15.0 statistical software.

RESULTS

Clinical characteristics of the patient and the control groups are described in Table 1. There were no significant differences between the two groups according to age, gender, BMI and systolic and diastolic blood pressure values. Fasting blood glucose levels and serum GH and IGF-1 levels were significantly higher in the patient group compared with the control group (pGlc: 0.001, pGH: 0.007, pIGF-1: 0.001, respectively). In the acromegaly group, 6 patients had type 2 diabetes and in the control group none of the subjects had diabetes. Lipid parameters were similar between the two groups (Table 2).

ABO blood groups which interfere in von Willebrand factor (vWF) and affect platelet activity, were similar between the two groups (p: 0.114).

Serum fibrinogen levels were significantly higher in acromegaly group (pfibrinogen: 0.011). In addition, acromegalic patients were found to have significantly enhanced platelet function as measured by collagen/ADP- and collagen/epinephrine- closure times. Collagen/ADP- and collagen/epinephrine-

Table 1. Clinical characteristics of the Study Population

	Acromegaly Group (n: 40)	Control Group (n: 36)	P value
Age	49.80 $\pm$ 12.59	46.92 $\pm$ 4.72	0.297
Gender (M/F)	19 / 21	16 / 20	0.487
BMI (kg/m ²)	29.11 $\pm$ 4.79	27.78 $\pm$ 5.41	0.162
Systolic blood pressure (mmHg)	121.96 $\pm$ 18.84	116.94 $\pm$ 10.85	>0.05
Diastolic blood pressure (mmHg)	86.74 $\pm$ 11.38	82.43 $\pm$ 6.97	>0.05

* $P < 0.05$.

Table 2. Laboratory parameters of the study population

	Acromegaly Group (n: 40)	Control Group (n: 36)	P value
Glucose (mg/dL)	109.73 $\pm$ 16.18	90.23 $\pm$ 8.72	0.001*
LDL-cholesterol (mg/dL)	116.84 $\pm$ 29.51	129.02 $\pm$ 28.55	0.095
HDL-cholesterol (mg/dL)	50.09 $\pm$ 12.48	54.38 $\pm$ 11.07	0.053
Total cholesterol (mg/dL)	194.84 $\pm$ 37.51	209.17 $\pm$ 40.42	0.137
Triglyceride (mg/dL)	145.18 $\pm$ 89.56	121.26 $\pm$ 63.85	0.237
GH (ng/mL)	4.02 $\pm$ 3.14	1.83 $\pm$ 3.31	0.007*
IGF-1 (ng/mL)	505.75 $\pm$ 215.03	124.27 $\pm$ 49.86	0.001*
Fibrinogen (mg/dL)	442.68 $\pm$ 146.36	362.51 $\pm$ 44.41	0.011*
Col/ADP-CT (sec)	82.94 $\pm$ 23.71	103.96 $\pm$ 41.33	0.015*
Col/Epi-CT (sec)	107.42 $\pm$ 19.18	124.20 $\pm$ 37.73	0.021*
MPV (fL)	9.45 $\pm$ 0.98	8.43 $\pm$ 0.99	0.001*
Platelet count (10 ³ /uL)	230.96 $\pm$ 55.84	265.29 $\pm$ 44.25	0.419

* $P < 0.05$.

Table 3. Baseline and final parameters of the Acromegaly Group

	Basal (n: 40)	Final (n: 40)	P value
BMI (kg/m ²)	29.11 ± 4.79	28.93 ± 4.08	0.223
Glucose (mg/dL)	109.73 ± 16.18	106.52 ± 14.84	0.024
LDL-cholesterol (mg/dL)	116.84 ± 29.51	121.66 ± 45.86	0.513
HDL-cholesterol (mg/dL)	50.09 ± 12.48	48.21 ± 13.74	0.505
Total cholesterol (mg/dL)	194.84 ± 37.51	197.08 ± 47.95	0.860
Triglyceride (mg/dL)	145.18 ± 89.56	158.74 ± 104.27	0.910
GH (ng/mL)	4.02 ± 3.14	3.44 ± 3.07	0.001*
IGF-1 (ng/mL)	505.75 ± 215.03	457.82 ± 182.49	0.010*
Fibrinogen (mg/dL)	442.68 ± 146.36	402.16 ± 153.18	0.021*
Col/ADP-CT (sec)	82.94 ± 23.71	97.68 ± 26.89	0.001*
Col/Epi-CT (sec)	107.42 ± 19.18	122.06 ± 32.37	0.028*
MPV (fL)	9.45 ± 0.98	9.04 ± 0.97	0.017*
Platelet count (10 ³ /uL)	230.96 ± 55.84	242.57 ± 64.55	0.667

*P<0.05.

closure times were significantly decreased in patients with acromegaly (pcol-ADP: 0.015, pcol-epinephrine: 0.021). We still observed similar findings when we excluded the diabetic patients in the acromegaly group (pfibrinogen: 0.011, pcol-ADP: 0.010, pcol-epinephrine: 0.011).

Although platelet count was similar between the two groups, acromegalic patients had significantly higher MPV values (pMPV: 0.001). Also, the result did not change when the diabetic patients were excluded (pMPV: 0.002).

All patients with acromegaly were re-evaluated 6 months after treatment (surgery and/or medical treatment). While fasting blood glucose levels decreased significantly (p: 0.024), lipid parameters remained similar. As expected, serum GH and IGF-1 levels decreased significantly (pGH: 0.001, pIGF-1: 0.010) after treatment. Likewise, serum fibrinogen levels and MPV values decreased significantly (pfibrinogen: 0.021, pMPV: 0.017). Collagen/ADP - and collagen/epinephrine - closure times were found to be significantly increased – indicating decreased platelet function - after 6-month treatment (pcol-ADP: 0.001, pcol-epinephrine: 0.028).

Among 38 acromegalic patients that were re-evaluated 6 months after surgery (two patients had refused to undergo surgery and were on long-acting somatostatin analog (SSA) treatment and still had active disease), 8 patients were in remission without adjuvant treatment (Group-1), 16 patients were in remission with long-acting SSA after surgery (Group-2) and 14 patients had still active disease (Group-3) despite of surgery and long-acting SSA therapy. When these three groups were compared, it was found that among all parameters evaluated, only serum fibrinogen, Collagen/ADP-closure times, MPV values were significantly

different between the three groups. Serum fibrinogen level was 325.57 ± 148.83 mg/dL in Group-1, 499.33 ± 156.97 mg/dL in Group-2 and 514.75 ± 45.74 mg/dL in Group-3 (p: 0.001). Collagen/ADP-closure time was 91.50 ± 29.49 sec in Group-1, 75.25 ± 19.50 sec in Group-2 and 71.61 ± 14.90 sec in Group-3 (p: 0.026). MPV values were 9.14 ± 0.72 fL in Group-1, 9.43 ± 1.35 fL in Group-2 and 9.51 ± 1.26 fL in Group-3 (p: 0.006). In comparison with the control group, it was found that patients in Group-1 had similar fibrinogen, MPV values and platelet function (p>0.05). However, patients in Group-2 had significantly higher fibrinogen levels and MPV values and decreased collagen/epinephrine-closure time with respect to the control group (pfibrinogen: 0.001, pMPV: 0.026, pcol-epinephrine: 0.037). Finally as expected, patients in Group-3 had significantly higher fibrinogen levels and MPV values and decreased collagen/ADP and collagen/epinephrine- closure times with respect to control group (pfibrinogen: 0.004, pMPV: 0.001, pcol-ADP: 0.032 and pcol-epinephrine: 0.045, respectively).

DISCUSSION

Cardiovascular and cerebrovascular events are the primary cause of death in acromegaly (5, 23). The increased prevalence of hypertension, insulin resistance, dyslipidemia, hypertrophic cardiomyopathy, and endothelial dysfunction in patients with acromegaly leads to increased rate of mortality. Among these risk factors, in our study fasting blood glucose levels were significantly higher in patients with acromegaly compared with the control group, but lipid profile and blood pressure values were similar in the two groups.

In addition to these risk factors, alterations in hemostatic parameters might also contribute to

the increased cardiovascular and cerebrovascular mortality in acromegaly patients. Fibrinogen is a well-known acute phase reactant and its metabolites can cause endothelial damage and dysfunction. It can initiate atherosclerotic plaque creation and accelerate its progression. Increased fibrinogen levels have been reported in patients with acromegaly (5-7, 9, 24). Similarly, in our study we observed increased fibrinogen levels in acromegalic patients. The effect of treatment (surgery and/or medical treatment) on fibrinogen levels in acromegalic patients was also evaluated previously (5, 6, 25, 26). Treatment or partial biochemical control of acromegaly (5, 6), six-month treatment with lanreotide (26) or octreotide LAR (25) significantly decreased fibrinogen levels. In concordance with these findings, we observed decrease in fibrinogen levels after treatment (surgery and/or medical treatment), but the decrease was significant only in patients who were in remission after surgery without adjuvant treatment. The increased fibrinogen levels may cause a tendency toward coagulation in patients with acromegaly and with the complete biochemical control of the disease -without the need for adjuvant therapy after surgery- this tendency for coagulation may be reversed.

Larger platelets contain more granules and produce greater amounts of vasoactive and prothrombotic factors, such as thromboxane A₂, serotonin and ATP; they aggregate more rapidly. Thromboxane A₂ causes vasoconstriction and vein occlusion. Increased MPV values are reported in various cardiovascular diseases (27, 28) and is a risk indicator for atherothrombosis. There are a few studies and conflicting results about MPV levels in acromegaly. While MPV values were found to be increased in the study of Unubol *et al.*, it was found to be similar with control groups in other studies (17, 19, 20). In our study, we found significantly higher MPV values in acromegalic patients with respect to controls. The effect of treatment on MPV values in acromegalic patients was also studied before (18-20), but the results were contradictory. While MPV values decreased significantly after treatment (surgery and SSAs) in one study, it was increased in another (20). Ucler *et al.* reported that MPV levels did not change in acromegalic patients controlled by surgery alone, but it was increased in patients controlled by both surgery and long-acting SSA therapy and they suggested that acromegalic patients receiving SSA treatment had an increased risk for atherothrombosis although the disease was under control. In our study, MPV values decreased significantly after treatment in patients who were in

remission after surgery without adjuvant therapy, but it remained unchanged in patients with active disease and in patients who were in remission with long-acting SSA after surgery. These results suggest that although acromegaly is controlled biochemically by SSA treatment, the risk of atherosclerosis may still remain increased.

Platelet activation is a hallmark of acute coronary syndrome. Increased platelet activation, that was evaluated by PFA-100 (platelet function analyser) which measured decreased collagen/ADP and collagen-epinephrine closure times, was previously reported in patients with acute coronary syndrome and myocardial infarction (8, 29). We previously investigated platelet function analysis in patients with acromegaly and reported decreased collagen/ADP and collagen-epinephrine closure times, indicating the platelet hyperfunction, in acromegalic patients with respect to controls. In the present study which included some of the acromegalic patients of the previous study, we observed significantly decreased Collagen/ADP- and collagen/epinephrine-closure times in patients with acromegaly in concordance with our previous findings. To our knowledge there is no study that investigated the effect of treatment on platelet function analysis in acromegalic patients. In this study, when we evaluated patients after six months of surgery, we found increased Collagen/ADP- and collagen/epinephrine-closure times -indicating decreased platelet function. But the increase was significant in patients who were in remission by only surgery. Even though the disease was controlled biochemically with SSA after surgery, the increased risk of hypercoagulability might not be improved.

In conclusion, our findings have shown that: patients with acromegaly had higher fibrinogen levels, MPV values and enhanced platelet function with respect to controls. Therefore, it may be suggested that, aforementioned changes in hemostatic parameters may lead to a hypercoagulable state which might increase the risk for cardiovascular and cerebrovascular events in acromegaly. Although growth hormone hypersecretion was controlled, these parameters did not recover completely. Therefore, in addition to acromegalic patients with active disease, biochemically controlled patients -especially by medical treatment after surgery-, should also be followed-up closely for cardiovascular risk factors and events.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

References

1. Vilar L, Naves LA, Costa SS, Abdalla LF, Coelho CE, Casulari LA. Increase of classic and nonclassic cardiovascular risk factors in patients with acromegaly. *Endocr Pract*. 2007;13(4):363-372.
2. Rajasoorya C, Holdaway IM, Wrightson P, Scott DJ, Ibbertson HK. Determinants of clinical outcome and survival in acromegaly. *Clin Endocrinol (Oxf)*. 1994;41(1):95-102.
3. Colao A, Marzullo P, Di Somma C, Lombardi G. Growth hormone and the heart. *Clin Endocrinol (Oxf)*. 2001;54(2):137-154.
4. Erem C, Nuhoglu I, Kocak M, Yilmaz M, Sipahi ST, Ucuncu O, Ersoz HO. Blood coagulation and fibrinolysis in patients with acromegaly: increased plasminogen activator inhibitor-1 (PAI-1), decreased tissue factor pathway inhibitor (TFPI), and an inverse correlation between growth hormone and TFPI. *Endocrine*. 2008;33(3):270-276.
5. Delaroudis SP, Efstathiadou ZA, Koukoulis GN, Kita MD, Farmakiotis D, Dara OG, Goulis DG, Makedou A, Makris P, Slavakis A, Avramides AI. Amelioration of cardiovascular risk factors with partial biochemical control of acromegaly. *Clin Endocrinol (Oxf)*. 2008;69(2):279-284.
6. Landin-Wilhelmsen K, Tengborn L, Wilhelmsen L, Bengtsson BA. Elevated fibrinogen levels decrease following treatment of acromegaly. *Clin Endocrinol (Oxf)*. 1997;46(1):69-74.
7. Sartorio A, Cattaneo M, Bucciarelli P, Bottasso B, Porretti S, Epaminonda P, Faglia G, Arosio M. Alterations of haemostatic and fibrinolytic markers in adult patients with growth hormone deficiency and with acromegaly. *Exp Clin Endocrinol Diabetes*. 2000;108(7):486-492.
8. Harrison P, Mackie I, Mathur A, Robinson MS, Hong Y, Erusalimsky JD, Machin SJ, Martin JF. Platelet hyper-function in acute coronary syndromes. *Blood Coagul Fibrinolysis*. 2005;16(8):557-562.
9. Colak A, Yilmaz H, Temel Y, Demirpence M, Simsek N, Karademirci I, Bozkurt U, Yasar E. Coagulation parameters and platelet function analysis in patients with acromegaly. *J Endocrinol Invest* 2016; 39(1): 97-101.
10. Greisenegger S, Endler G, Hsieh K, Tentschert S, Mannhalter C, Lalouschek W. Is elevated mean platelet volume associated with a worse outcome in patients with acute ischemic cerebrovascular events? *Stroke* 2004, 35(7):1688-1691.
11. Gursoy A, Ertugrul DT, Pamuk B, Sahin M, Asik M, Yilmaz H, Haydardedeoglu F, Tutuncu NB, Demirag NG. Mean platelet volume in patients with polycystic ovary disease. *Platelets* 2006, 17(7):505-506.
12. Brown AS, Hong Y, de Belder A, Beacon H, Beeso J, Sherwood R, Edmonds M, Martin JF and Erusalimsky JD. Megakaryocyte Ploidy and Platelet Changes in Human Diabetes and Atherosclerosis. *Arterioscler Thromb Vasc Biol* 1997, 17(4):802-807.
13. Endler G, Klimesch A, Sunder-Plassmann H, Schillinger M, Exner M, Mannhalter J, Jordanova N, Christ G, Thalhammer R, Huber K and Sunder-Plassmann R. Mean platelet volume is an independent risk factor for myocardial infarction but not for coronary artery disease. *Br J Haematol* 2002, 117(2):399-404.
14. Kaya MG, Yarlioglu M, Gunebakmaz O, Gunturk E, Inanc T, Dogan A, Kalay N and Topsakal R. Platelet activation and inflammatory response in patients with non-dipper hypertension. *Atherosclerosis* 2010, 209(1):278-282.
15. Çoban E, Yazıcıoğlu G, Özdoğan M. Platelet activation in subjects with subclinical hypothyroidism. *Med Sci Monit* 2007, 13(4):211-214.
16. Erikci AA, Karagoz B, Ozturk A, Caglayan S, Ozisik G, Kaygusuz I, Ozata M. The effect of subclinical hypothyroidism on platelet parameters. *Hematology* 2009, 14(2):115-117.
17. Unubol M, Guncy E, Ture M, Eryilmaz U. Mean platelet volume and arterial stiffness in patients with acromegaly. *Anadolu Kardiyol Derg*. 2014 Aug;14(5):456-463.
18. Ucler R, Aslan M, Atmaca M, Alay M, Ademoglu EN, Candan Z, Gulsen I. The effect of disease control on mean platelet volume and red blood cell distribution in patients with acromegaly. *Int J Clin Exp Med* 2015; 8(4): 6060-6066.
19. Ersoy R, Gul K, Solaroglu N, Dirikoc A, Aydin C, Soydal C, B. Cakir. Effect of a six-month treatment with octreotide long acting repeatable (LAR) on mean platelet volume in patients with acromegaly. *Endocrine Abstracts* 2008; 16:431.
20. Durmaz SA, Carlioglu A, Ayhan E, Macunluoglu B. The effects of octreotide LAR on mean platelet volume in acromegaly; octreotide LAR may have a detrimental effect on MPV, a new indicator of atherosclerosis. *Endocrine Abstracts* 2014; 35:855.
21. Jilma B. Platelet function analyzer (PFA-100): a tool to quantify congenital or acquired dysfunction. *J Lab Clin Med* 2001; 138(3): 152-163.
22. Katznelson L, Laws ER Jr, Melmed S, Molitch ME, Murad MH, Utz A, Wass JA. Endocrine Society. Acromegaly: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2014 Nov;99(11):3933-3951.
23. Mestron A, Webb SM, Astorga R, Benito P, Catala M, Gaztambide S, Gomez JM, Halperin I, Lucas-Morante T, Moreno B, Obiols G, de Pablos P, Paramo C, Pico A, Torres E, Varela C, Vazquez JA, Zamora J, Albareda M, Gilabert M. Epidemiology, clinical characteristics, outcome, morbidity and mortality in acromegaly based on the Spanish Acromegaly Registry (Registro Espanol de Acromegalia, REA). *Eur J Endocrinol*. 2004 Oct;151(4):439-446.
24. Kaluzny M, Bolanowski M, Daroszewski J, Szuba A. The role of fibrinogen and CRP in cardiovascular risk in patients with acromegaly. *Endokrynol Pol*. 2010; 61(1):83-88.
25. Potter BJ, Beauregard C, Serri O. Serum markers of cardiovascular risk in patients with acromegaly before and after six months of treatment with octreotide LAR. *Pituitary* 2008; 11(1): 49-53.
26. Colao A, Marzullo P, Lombardi G, and the Italian Multicenter Study Group on Lanreotide. Effect of six-month treatment with lanreotide on cardiovascular risk factors and arterial intima-media thickness in patients with acromegaly. *Eur J Endocrinol* 2002; 146(3): 303-309.
27. Vizioli L, Muscari S, Muscari A. The relationship of mean platelet volume with the risk and prognosis of cardiovascular diseases. *Int J Clin Pract* 2009, 63(10):1509-1515.
28. Pizzulli L, Yang A, Martin JF and Lüderitz B. Changes in platelets size and count in unstable angina compared to stable angina or non cardiac chest pain. *Eur Heart J* 1998, 19(1):80-84.
29. Frossard M, Fuchs I, Leitner JM, Hsieh K, Vlcek M, Losert H, Domanovits H, Schreiber W, Laggner AN, Jilma B. Platelet function predicts myocardial damage in patients with acute myocardial infarction. *Circulation*. 2004;110(11):1392-1397.