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Original/*Obesidad*

Biomarkers of the prothrombotic state in abdominal obesity

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Abstract

Introduction: **Central obesity is specifically associated with cardiovascular disease. Nevertheless, the molecular events that promote these conditions remain incompletely defined and risk stratifying patients for cardiovascular disease continues a challenge.**

Objective: **The aim of this study was to assess some cost-efficient haemostatic markers, and its association with central obesity and traditional cardiovascular risk factors, in a cohort of middle aged subjects, without clinical cardiovascular disease, as basis for an improved prevention and intervention.**

Methods: **We studied 307 men, aged 45±7 years, which underwent medical history, physical examination, anthropometric measurements, plasmatic biochemical parameters, plasma concentrations of fibrinogen, prothrombin activity, activated partial thromboplastin time, platelet count and mean platelet volume.**

Results: **Prothrombin activity values were significantly higher in patients with central obesity (103 ± 16 % vs 111 ± 17 %, p<0.001). Across tertiles of fibrinogen (low and high), there was an increase in cholesterol, adjusted for age and body mass index (4.9±0.9 mmol/L vs 5.4±1.1 mmol/L, p< 0.01). High tertile of prothrombin activity showed higher levels of cholesterol (4.8±1.0 mmol/L vs 5.4±0.9 mmol/L , p< 0.05), triglycerides (1.07±0.6 mmol/L vs 1.32±0.9 mmol/L, p< 0.05), and waist circumference (92.8±8.3 cm vs 96.5±8.8 cm, p= ns) . Mean values of cholesterol were higher in low-activated partial thromboplastin time tertile (5.3±0.9 mmol/L vs 4.9±1.1 mmol/L, p<0.01). Participants in the high-mean platelet volume tertile showed higher levels of glycemia (5.7±0.6 mmol/L vs 5.99±0.7 mmol/L, p<0.05). Significant positive correlations were observed between fibrinogen and cholesterol (r=0.198, p<0.001) and triglycerides (r=0.116, p<0.05). Prothrombin activity was positively correlated with waist circumference (r=0.156, p<0.05), glucose (r=0.227, p<0.001), cholesterol (r=0.270, p=0.001), triglycerides (r=0.187, p=0.001) and mean platelet volume (r=0.130, p=0.05). Activated partial thromboplastin time was inversely related cholesterol (r=-0.172, p<0.01) concentra-**

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BIOMARCADORES DE ESTADO PROTROMBÓTICO EN LA OBESIDAD ABDOMINAL

Resumen

Introducción: **La obesidad central está especialmente asociada con la enfermedad cardiovascular. No obstante, los mecanismos moleculares que la promueven no están completamente definidos y la estratificación del riesgo de los pacientes para la enfermedad cardiovascular sigue siendo un reto.**

Objetivo: **Evaluar la asociación de la adiposidad central con marcadores hemostáticos coste-eficientes y con los factores de riesgo cardiovascular tradicionales en una cohorte de varones de mediana edad sin enfermedad cardiovascular clínica con objeto de mejorar la prevención y la intervención.**

Métodos: **Estudiamos 307 varones de 45±7 años, a los que se realizó historia clínica, examen físico, mediciones antropométricas y determinaciones plasmáticas bioquímicas y de fibrinógeno, actividad protrombina, tiempo de tromboplastina parcial activada, recuento de plaquetas y volumen plaquetario medio.**

Resultados: **Los valores de actividad protrombina fueron significativamente más elevados en pacientes con obesidad central (103 ± 16 % vs 111 ± 17 %, p<0.001). Los sujetos con cifras de fibrinógeno en el tertil alto presentaban un aumento en las concentraciones plasmáticas de colesterol (4.9±0.9 mmol/L vs 5.4±1.1 mmol/L, p< 0.01), ajustado por edad e índice de masa corporal. El tertil alto de actividad protrombina mostró concentraciones más elevadas de colesterol plasmático (4.8±1.0 mmol/L vs 5.4±0.9 mmol/L, p< 0.05), triglicéridos (1.07±0.6 mmol/L vs 1.32±0.9 mmol/L, p< 0.05), and medida de la circunferencia de la cintura (92.8±8.3 cm vs 96.5±8.8 cm, p= ns) . Las concentraciones medias de colesterol fueron más altas en el tertil bajo de tiempo de tromboplastina parcial activado (5.3±0.9 mmol/L vs 4.9±1.1 mmol/L, p<0.01). Los individuos en el tertil alto de volumen plaquetario medio presentaron glucemia más elevada (5.7±0.6 mmol/L vs 5.99±0.7 mmol/L, p<0.05). Se observaron correlaciones significativas positivas entre fibrinógeno y colesterol (r=0.198, p<0.001) and triglicéridos (r=0.116, p<0.05). La actividad protrombina estuvo positivamente correlacionada con la medida de la circunferencia de la cintura (r=0.156, p<0.05), glucemia (r=0.227, p<0.001), colesterol (r=0.270, p=0.001), triglicéridos (r=0.187, p=0.001) y volumen plaquetario medio (r=0.130, p=0.05). El tiempo de tromboplastina parcial activado estuvo inversamente relacionado con las concentraciones de colesterol (r=-0.172, p<0.01). El volumen plaquetario medio se correlacionó con las concentraciones de glucosa (r=0.170, p<0.01).**

tions. Mean platelet volume rose with increasing glucose concentrations (r=0.170, p<0.01).

Conclusions: **Haemostatic markers studied have shown association with abdominal adiposity and established cardiovascular risk factors. These markers are widely available, relatively inexpensive, and might allow risk stratifying patients for cardiovascular disease and the identification of hypercoagulable state in patients who might deserve preventive measures and are potential tools for assessing the impact of these measures.**

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Key words: *Obesity. Cardiovascular diseases. Fibrinogen. Prothrombin.*

Conclusiones: **Los marcadores hemostáticos estudiados se asocian con la adiposidad abdominal y con los factores de riesgo cardiovascular establecidos. Estos marcadores están disponibles en clínica, son asequibles y podrían ser de ayuda en la estratificación del riesgo cardiovascular de los pacientes y en la identificación del estado hipercoagulable en personas que podrían beneficiarse de medidas preventivas. Además de ser herramientas potenciales para evaluar el impacto de dichas medidas.**

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Palabras Clave: *Obesidad. Enfermedad cardiovascular. Fibrinógeno. Protrombina.*

Abbreviations

PT: Prothrombin activity.
APTT: Activated partial thromboplastin time.
MPV: Mean platelet volume.
BMI: Body mass index.
WC: Waist circumference.

Introduction

Obesity has reached epidemic proportions in Western societies and is a strong risk factor for the development of cardiovascular disease. Central obesity is specifically associated with cardiovascular mortality and obesity is a significant risk factor for the development of arterial thrombosis and venous thromboembolism. Nevertheless, the molecular events that promote these conditions remain incompletely defined and risk stratifying patients for cardiovascular disease remains a challenge because many patients identified to be at low or intermediate risk experience a cardiovascular event[1, 2].

For these reasons there has been considerable interest in identifying biomarkers that might improve the global risk prediction of cardiovascular disease. Among these, some haemostatic markers can be highlighted, such as fibrinogen, prothrombin activity (PT), activated partial thromboplastin time (APTT) and platelet activity measuring, like platelet count and mean platelet volume (MPV).

Fibrinogen, an acute phase reactant, is synthesized in the liver. It is an important component of the coagulation pathway and a major determinant of plasma viscosity[3].

Clinical studies yield strong evidence that fibrinogen levels are increased in obese and overweight individuals with abdominal fat distribution. A positive correlation has been observed between body index mass (BMI), insulin resistance, metabolic syndrome and fibrinogen[4].

The PT an APTT are globally accepted parameters for assessing the activity of extrinsic and intrinsic coagulation pathways[5]. The clinical significance of short APTT has gained interest recently, since it might be considered as a marker for hypercoagulability. A shortened APTT has been related to an increase in thrombin generation, chest pain, acute myocardial infarction and risk of thrombosis[6].

On the other hand, platelets play a pivotal role in atherothrombosis. Activation of platelets at the site of vascular injury is central to the pathogenesis of occlusive arterial disease. Studies on platelet function have aroused interest since the advent of novel treatment modalities such as antiplatelet drugs and stents[7].

Platelet count and MPV are markers of platelet activation widely available in clinical practice. Mean platelet volume is a potential marker of platelet reactivity. Larger platelets are younger, more reactive and have greater prothrombotic potential. Furthermore, higher MPV is observed in patients with diabetes mellitus, hypertension, hypercholesterolemia, smoking and obesity[8]. Although measuring platelet activity has been reported to identify individuals at increased risk for cardiovascular events, it remains a research tool that yet to be included in routine clinical decisions-making.

Objective

The aim of this study was to assess some cost-efficient hemostatic markers, such as plasma levels of fibrinogen, PT, APTT, platelet count and MVP, and its association with central obesity and traditional cardiovascular risk factors, in a cohort of middle aged subjects, without clinical cardiovascular disease, as basis for an improved prevention and intervention.

Methods

Study Population

This is a cross-sectional study involving 307 men from San Carlos Hospital, Cádiz, that attend employment medical examination, undertaken from March

to October 2011. The sample size was set in order to provide a specific relative precision of 3%, a 95% confidence interval, taking into account 5% of MVP ≥11.7 fL prevalence. All subjects were free of clinical acute or chronic disease in the medical record.

Anthropometry, blood pressure, health behaviors, hematology and biochemistry

Measurements were performed as described: height was determined using a mobile stadiometer (model Seca 217, Hamburg, Germany), body weight was recorded by a digital scale (model Seca 876, Hamburg, Germany), both measures were used to calculate body mass index (kg/m^2). Waist circumference (WC) was measured in a standing position, midway between the lower costal margin and the iliac crest, using a 2-metre non-stretch fiberglass tape. Central obesity was defined as a waist circumference ≥ 94 cm. [9] Resting blood pressure was measure on the right arm, at the end of the physical examination with the participant sitting, using mercury sphygmomanometer. Smoking status (number of cigarettes per day), alcohol intake (g/day) and physical activity (exercise: number of hours per day) were evaluated by structured interview. We used the SCORE chart for cardiovascular risk estimation[9]. The following parameters were measured in fasting blood samples: glucose (plasma), cholesterol, triglycerides, uric acid (serum), platelet count and MPV (whole blood, EDTA anticoagulant). Fibrinogen, PT and APTT were assessed by an analyzer (STA compact- Diagnostica Stago, Asnieres, France) using the appropriate reagents (Roche Diagnostics, Basel, Switzerland)[10,11].

Statistical Analysis

Data analysis was performed using SPSS v19.0 (SPSS Inc., Chicago, IL, USA). If variables were not normally distributed, they were logarithmically transformed. Associations between risk factors and fibrinogen, PT, APTT, platelet count and MPV were assessed by grouping participants into tertiles of fibrinogen, PT, APTT, platelet count and MPV (≤33th, 34-65th and ≥ 66th percentile). Means were compared by an ANOVA test or by Kruscal-Wallis test depending on the distribution of the data. A value of $p<0.05$ was considered statistically significant. Spearman´s correlation test and partial correlation coefficients were performed to analyze the linear relationship between variables.

Ethics

The study was approved by the ethics committee of our university and informed consent was obtained. The investigation conforms with the principles outlined in the Declaration of Helsinki.

Results

Characteristics of participants and cardiovascular risks factors are presented in Table I. Prothrombotic biomarkers of the patients with and without abdominal obesity are presented in Table II. Prothrombin activity values were significantly higher in patients with central obesity (103 % ± 16 % vs 111 % ± 17 %, $p<0.001$). There were no statistically significant differences between the two groups with respect to fibrinogen, APTT, platelet count and MPV. Across tertiles of fibrinogen (low and high), there was an increase in cholesterol (the difference remained significant after adjusting for age and BMI) (4.9±0.9 mmol/L vs 5.4±1.1 mmol/L, $p< 0.01$), PT activity (100±22 % vs 111±19 %, $p< 0.001$) and a decrease in APTT (35±11 s vs 32±5 s**,** $p<0.01$) (Table III).

High tertile of PT showed higher levels of cholesterol (4.8±1.0 mmol/L vs 5.4±0.9 mmol/L **,** $p< 0.05$),

Table I
Characteristics of the study subjects

N = 307	*Mean±SD*
Age (years)	45.2±7
WC (cm)	94.8±9.2
BMI (kg/m^2)	27.5±3.5
SBP (mmHg)	124±14
DBP (mmHg)	71±10
Smoking (nºc/d)	4.0±8.2
Smoking (%)	26 %
Exercise (nº h/week)	1.3±1.4
Any alcohol intake (%)	68%
Any alcohol intake (g/d)	10±10
Glucose (mmol/L)	5.83±0.83
Cholesterol (mmol/L)	5.18±1.06
Triglycerides (mmol/L)	1.22±0.88
Uric acid (μmol/L)	333±65
Fibrinogen (g/L)	3.1±0.8
PT (%)	106±20
PT (s)	13.2±3.1
PT(INR)	1.04±0,77
APTT (s)	34±9
Platelets count (nº /mm^3)	226±49
MPV (fL)	10.7±0.8
SCORE (%)	0.91±1.35

Waist circumference, WC; body mass index, BMI; Systolic blood pressure, SBP; Diastolic blood pressure, DBP; Prothrombin time, PT; activated partial thromboplastin, APTT; Mean platelet volume, MPV.

Table II
Hemostatic biomarkers in normal men and in men with abdominal obesity (WC ≥94)

Variable	*WC < 94*	*WC ≥94*
n	175	132
Fibrinogen	3.0±0.7	3.2±0.8
PT	**103± 16**	**111± 17***
APTT	34±10	34±11
Platelet count	225±45	231±51
MPV	10,6±0,9	10,7±0,8

Results are expressed as mean ± standard deviation. Fibrinogen (g/L); Prothrombin, PT (activity %);
Activated partial thromboplastin, APTT(s); Mean platelet volume, MPV (fL); Platelet count (nº/mm³).
Statistically significant: ANOVA *p< 0.001.

triglycerides (1.07±0.6 mmol/L vs 1.32±0.9 mmol/L, p< 0.05) and fibrinogen (2.94±0.86 g/L vs 3.36±0.93 g/L, p< 0.001), adjusted for age and BMI, and lower of APTT (p< 0.001) and higher WC (92.8±8.3 cm vs 96.5±8.8 cm, p= ns) .

Mean values of fibrinogen (3.24±0.77 g/L vs 3.00±0.84 g/L, p<0.001), cholesterol (5.3±0.9 mmol/L vs 4.9±1.1 mmol/L**,** p<0.01) and PT (113±14 % vs 100±27%, p< 0.001), adjusted for age and BMI, were higher in low-APTT tertile.

Participants in the high-MPV tertile showed higher levels of glycemia (5.7±0.6 mmol/L vs 5.99±0.7 mmol/L, p<0.05) and lower platelet count (251 ± 53 / mm³ vs 196 ±36 /mm³, p<0.001), after adjusting for age and BMI (Table IV).

There were no statistically significant differences between tertiles of prothrombotic biomarkers with respect to BMI and WC, although WC was higher in high-PT tertile, as already mentioned.

Table III
Cardiovascular risk markers across tertiles of fibrinogen, PT and APTT

	Fibrinogen		*PT*		*APTT*	
	Low	**High**	**Low**	**High**	**Low**	**High**
	≤267	≥332	≤100	≥113	≤31.4	≥34.3
n	103	103	105	102	102	103
Age	44±6	46±7	44±7	45±7	46±7	44±6
BMI	27.3±3.5	27.8±3.6	27.1±3.5	27.5±3.3	27.7±3.3	27.5±4.3
WC	94.2±9.0	96.3±8.9	92.8±8.3	96.5±8,8	95.2±8.3	95.3±11.7
SBP	125±13	123±14	123±13	125±14	124±15	124±12
DBP	72±11	71±10	71±10	73±10	70±11	72±9
Smoking	3.3±6.9	5. 6±9.4	4.4±8.8	4.9±9.4	4.5±9.4	4.2±8.3
Exercise	1.2±1.4	1.1±1.4	1.4±1.6	1.0±1.2	1.4±1.5	1.0±1.3
Alcohol	12±11	9±9	9±9	11±10	10±10	9±11
Glucose	5.8±1	5.8±0.8	5.7±1.1	5.9±0.6	5.9±1.0	5.7±0.5
Cholesterol	**4.9±0.9**	**5.4±1.1****	**4.8±1.0**	**5.4±0.9***	**5.3±0.9**	**4.9±1.1****
TG	1.13±0.8	1.28±1.1	**1.07±0.6**	**1.32±0.9***	1.12±0.9	1.28±0.9
Uric acid	327±59	339±77	315±59	344±77	344±71	327±65
Fibrinogen	2.36±0.27	4.05±0.74	**2.94±0.86**	**3.36±0.93*****	**3.24±0.77**	**3.00±0.84*****
PT	**100±22**	**111±19*****	88±16	125±14	**113±14**	**100±27*****
APTT	**35±11**	**32±5****	**37±14**	**31±4*****	29.3±1.4	40.3±14
Platelet	223±45	227±50	236±52	218±46	222±52	229±51
MPV	10.5±0.9	10.8±0.9	**10.4±0.8**	**10.8±0.8****	10.7±0.9	10.7±0.9
SCORE	0.73±1.05	1.14±1.40	0.79±1.2	1.14±1.78	1.21±1.68	0.60±0.80

Results are expressed as mean ± standard deviation. Waist circumference, WC; Body mass index, BMI; Systolic blood pressure, SBP; Diastolic blood pressure, DBP; Smoking (nºc/d); Exercise (nº h/week); Alcohol intake (g/d); .Glucose (mmol/L); Cholesterol (mmol/L); Triglycerides, TG (mmol/L); Uric acid (μmol/L); Fibrinogen (g/L); Prothrombin time, PT (activity %); Activated partial thromboplastin, APTT(s); Mean platelet volume, MPV (fL); Platelet count (nº/mm³); SCORE (%). Statistically significant: (ANOVA adjusted for age and BMI: Cholesterol, Triclycerides, Fibrinogen, MPV). (Kruskal-Wallis: PT, APTT). * p<0.05, **p<0.01, ***p<0.001.

Tabla IV
Cardiovascular risk markers across tertiles of platelet count and MPV

	Platelet count		*MPV*	
	Low	**High**	**Low**	**High**
	≤203	≥241	≤10.2	≥11
n	103	103	101	102
Age	44±7	45±7	45.4±7.4	45.4±7.4
BMI	27.3±3.5	27.7±3.9	27.5±3.8	27.1±3.1
WC	94.0±9.4	95.1±8.7	94.2±9.1	93.6±8.0
SBP	122±13	123±14	125±15	122±13
DBP	69±10	71±10	70±11	69±10
Smoking	3.7±6.9	5.8±10.7	5.1±9.4	4.0±8.1
Exercise	1.4±1.5	1.0±1.4	**1.0±1.3**	**1.6±1.6 ***
Alcohol	9±9	10±9	10±12	10±8
Glucose	5.8±0.5	5.8±1.0	**5.7±0.6**	**5.99±0.7 ***
Cholesterol	5.2±1.1	5.1±1.0	5.1±0.9	5.2±1.1
TG	1.24±1.0	1.2±0.8	1.12±0.6	1.38±1.0
Uric acid	327±59	344±71	321±59	327±65
Fibrinogen	3.11±0.78	3.18±0.95	3.01±0.83	3.20±0.87
PT	107±23	104±19	105±20	107±20
APTT	34±11	34±10	105±20	107±20
Platelet	176±18	280±35	**251±53**	**196±36 *****
MPV	**11.0±1.4**	**10.1±1.2 *****	9.7±0.3	11.7±0.5
SCORE	0.92±1.69	0.92±1.20	1.14±1.86	0.86±1.09

Results are expressed as mean ± standard deviation. Waist circumference, WC; Body mass index, BMI; Systolic blood pressure, SBP; Diastolic blood pressure, DBP; Smoking (nºc/d); Exercise (nº h/week); Alcohol intake (g/d); Glucose (mmol/L); Cholesterol (mmol/L); Triglycerides, TG (mmol/L); Uric acid (μmol/L); Fibrinogen (g/L); Prothrombin time, PT (activity %); Activated partial thromboplastin, APTT(s); Platelet count (nº/mm^3); Mean platelet volume, MPV (fL); SCORE (%).Statistically significant (ANOVA adjusted for age and BMI: Glucose, Platelet count, MPV). (Kruskal-Wallis: exercise). * p<0.05, **p<0.01, ***p<0.001.

Significant positive correlations were observed between fibrinogen and age, cholesterol (r=0.198, p<0.001) and triglycerides (r=0.116, p<0.05). With respect to cholesterol concentrations, the correlation remained significant after controlling for age and BMI (r=0.193, p=0.01). PT was positively correlated with WC (r=0.156, p<0.05), glucose (r=0.227, p<0.001), cholesterol (r=0.270, p=0.001), triglycerides (r=0.187, p=0.001) and MPV (r=0.130, p=0.05). APTT was inversely related to age and cholesterol (r=-0.172, p<0.01) concentrations. MPV rose with increasing glucose concentrations, controlling for age and BMI, (r=0.170, p<0.01) and platelet count was inversely related to MPV (Table V). None of the outcomes was related to tobacco use or alcohol intake, except higher exercise in high MPV-tertile (1.0±1.3 vs 1.6±1.6 p<0.05).

Discussion

The current study analyzed the complex interactions between haemostatic markers, platelet activity indicators, obesity and established cardiovascular risks factors, trying to provide scientific and clinically relevant information, in order to establish novel strategies in the prevention and treatment of atherothrombosis.

Mean fibrinogen levels (3.1±0.8 g/L) were similar to those reported for subjects without clinical cardiovascular disease [12] and lower than those found in high cardiovascular risk population or patients with diabetes type 2[13,14].

Fibrinogen concentration was not positively related to abdominal adiposity in our study. A recently published article showed fibrinogen association with overweight and trunk obesity [15], although this associa-

Tabla V
Correlation test between established cardiovascular risk factors and haemostatic biomarkers

Variable	*Significant correlations*
Fibrinogen	Age (r=0.148, p<0.05), cholesterol (r=0.198, p<0.001), triglycerides (r=0.116, p<0.05), PT % (r=0.271, p<0.001), APTT (r= -0.195, p<0.001). *Cholesterol (r=0.193, p<0.01).
PT %	WC (r=0.156, p<0.05), glucose (r=0.227, p<0.001), cholesterol (r=0.270, p<0.001), triglycerides (r=0.187, p<0.001), fibrinogen (r=0.271, p<0.001), APTT (r=-0.386, p<0.001), MPV (r=0.130, p<0.05).
APTT	Age (r=-0.154, p<0.05), cholesterol (r=-0.172, p<0.01), fibrinogen (r=-0.195, p<0.001), PT % (r=-0.386, p<0.001), SCORE (r=-0.177, p<0.01).
Platelet count	MPV (r=-0.458, p<0.001). *MPV (r=-0.529, p<0.001).
MPV	Exercise (r=0.176, p<0.01), glucose (r=0.177, p<0.01). *Glucose (r=0.170, p<0.01).

Spearman's correlation coefficients. * Partial correlation coefficients controlling for age and body mass index. Waist circumference, WC; Exercise (nº h/week); Glucose (mmol/L); Cholesterol (mmol/L); Triglycerides, TG (mmol/L); Fibrinogen (g/L); Prothrombin time, PT (activity %); Activated partial thromboplastin, APTT(s); Mean platelet volume, MPV (fL); Platelet count (nº/mm3); SCORE (%).

tion was higher in women[16] and our participants were all men. Consistent with DRUID study cigarette smoking was not significantly associated with fibrinogen.[13]

Cholesterol rose with increasing fibrinogen levels (p=0.007) (Table III) and fibrinogen levels have been correlated with early atherosclerotic changes of the carotid artery in young healthy adults with mean fibrinogen concentrations about 2.6 ± 0.05 g/L.[12] These fibrinogen levels are lower than those found in our participants with mean age 45±7 years. It has been suggested that in subjects with very low cardiovascular risk, fibrinogen levels might serve as early and highly valid predictors for the development of atherosclerotic disease[17].

Different mechanisms have been identified by which fibrinogen might promote atherosclerosis. Fibrinogen can bind to endothelial cell receptors (ICAM-1) and trigger the release of vasoactive mediators. Fibrinogen has been shown to modulate endothelial cell permeability and smooth muscle cell proliferation.[18] We found relationship between high cholesterol levels and increased PT activity and shortened APTT (Table 3 and 5). Other studies attribute a potential role to shortened APTT as haemostatic marker of hypercoagulable state and it could be a useful screening test applicable to clinical conditions[19].

In our work PT was associated with abdominal obesity, glucose concentrations and triglycerides; and APTT with age and cholesterol. Lippi et al. showed that APTT values below the reference range (< 25.5 s) were significantly more frequent in diabetics than in normal subjects. In contrast to their findings, our results showed higher mean values of APTT (34.1±9.3 s) and they were not associated with glycemia. This could be explained by the lower age of our participants (45±7 years) compared to those of Lippi et al. (61 years).[6] However, since PT and APTT are relatively inexpensive tests, it might allow the identification of a subset of patients at major risk of thromboembolic complications who deserve more aggressive antithrombotic preventive measures.

Previous studies have reported increased fibrinogen levels with aging[12, 13] and we observed significant correlation between age and haemostatic markers, fibrinogen and APTT. Although, in a recent experimental study, no age-dependent alteration in PT and APTT was recorded, suggesting comparable function among young and old mice[5]. The authors of this study propose that aging per se does not affect arterial thrombosis and that age-dependent vascular dysfunction in the absence of additional risk factors does not alter arterial thrombus formation. These findings underscores the importance of controlling modifiable risk factors in aged individuals.

Regarding platelet activity, our findings of MPV values (10.7±0.8 fL) were similar to those of others authors in healthy subjects who suggest that patients having a MPV beyond 11.7 fL should be evaluated for occlusive arterial diseases[20]. This was the average MPV value of the high tertile in our study, in which we observed elevated levels of glucose. Mean platelet volume is simple, inexpensive, easy to interpret and routinely measured by automated cell counters. As compared with other markers of platelet activity, which require specialized equipment, MPV is a practical and prognostically important biomarker of cardiovascular disease[8].

Our findings did not show significant differences between tertiles of MPV with regard to BMI and abdominal adiposity. However, in contrast to our results others studies have reported this association. Furthermore, platelet function is modulated by several key regulators of body weight and metabolism. For example, platelet-dependent thrombosis is increased by leptin,

and is decreased by adiponectin. One explanation for this could be the presence of the leptin receptor on platelets, which acts synergistically with ADP to promote platelet aggregation[21-23].

Accordingly, obesity is associated with increased platelet activation, an event that has implications for both thrombosis and inflammation. The inflammatory response related to fat accumulation may influence cardiovascular risk through its involvement in coagulation and atherosclerosis. Thus, clinical markers of a prothrombotic state may indicate a risk for development of complications of insulin resistance or metabolic syndrome in obese or not obese[24, 25].

Although a significant amount of data has emerged demonstrating an association of MPV with myocardial infarction and death in patients with established coronary artery disease, there is much less data in patients without coronary artery disease. In a prospective cohort study of 25,923 apparently healthy men and women followed up for more than 10 years, subjects with an MPV ≥ 9.5 were 50% more likely to develop a venous thromboembolism as compared with those with MPV < 8.5[26]. In line with these findings, a large majority of individuals in our study would be in the range of risk (mean: 10.7±0.8 fL). Additionally, a study of more than 39,000 subjects from the Danish general population demonstrated that higher MPV was associated with incident myocardial infarction and all-cause mortality[27].

A recently published meta-analysis showed that MPV was higher in patients with acute myocardial infarction (MPV = 9.2 fL) than in those without acute myocardial infarction (MPV = 8.5 fL). [8] This analysis supports the hypothesis that platelet size may play a role in both the development and consequences of cardiovascular disease. Moreover, prognostic value of MPV has been validated in patients with decompensated heart failure (MPV > 10.5 fL) and pulmonary embolism (MPV > 10.9 fL)[28,29]. It is likely that an increased MPV predates an acute myocardial infarction rather than results from it. As the average platelet lifespan in blood is 7-10 days, the vast majority of platelets sampled at the time of hospital admission for an acute myocardial infarction would have been circulating before the event. Recent genome-wide studies have identified loci involved with MPV and it is being studied the relationship between genetic determinants of MPV and cardiovascular phenotype[30].

In clinical practice, increased MPV may be of value to detect high-risk patients for future cardiovascular events and can be used as a diagnostic or prognostic measurements. The assessment of MPV could be useful for identifying patients who would benefit from antiplatelet therapy and prediction of ischemic events. It is known that clopidogrel significantly inhibits the ADP-induced increase in MPV and aspirin has no effect on platelet size[7]. Also, obesity is thought to cause resistance to antiplatelet therapy[24].

Regarding platelet count, we observed an inverse association with MPV and consistent with other reports, platelet count was not associated with cardiovascular risks factors[31, 32]. However, both measures were inversely related and high tertile of MPV showed higher glucose concentrations (adjusted for age and BMI) suggesting that they could play a role as possible risk indicator independent of plasma lipids and adiposity.

Study limitations

There were certain limitations in this study: First, the cross-sectional design with healthy men. It will be interesting to follow up the participants and to examine aging-related effects on haemostatic system in future investigations. Second, preanalytical factors such as anticoagulant used, timing of blood sample preparation and calibration of particle counters, which may have an impact on the measurement of MPV. However, as sample preparations did not vary within the study because all sample were processed in the same laboratory, we do not believe that this limitation significantly altered our results or the conclusions that should be drawn from them.

Conclusions

In conclusion, haemostatic factors studied have shown association with abdominal adiposity and established cardiovascular risk factors such as, plasma cholesterol and triglycerides, indicating that could be markers of cardiovascular risk. Mean platelet volume was significantly higher in subjects with increased prothrombin activity, showing potential prothrombotic effect. Also, subjects grouped in the highest tertile of MPV presented values that are considered predictive of cardiovascular disease. Since MPV is routinely measured as a platelet standardized parameter, we suggest MPV is a potentially useful cardiovascular risk biomarker. Haemostatic tests studied are widely available, relatively inexpensive, and might allow risk stratifying patients for cardiovascular disease and the identification of hypercoagulable state in patients who might deserve preventive measures. Finally, there is evidence that the lifestyles that promote weight reduction, smoking cessation and physical exercise, decrease platelet reactivity and coagulability and stimulate fibrinolysis. This translates into improved cardiovascular prognosis and therefore, haemostatic parameters studied are potential tools for assessing the impact of these measures.

Conflict of interest

The authors declare no potencial conflicts of interest with respect to the research, authorship and /or publication of this article.

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