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Angiogenesis, inflammation and endothelial function in postmenopausal women screened for the metabolic syndrome

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ABSTRACT

Background: Prevalence of the metabolic syndrome (METS) increases after the menopause; nevertheless, concomitant vascular, inflammatory and endothelial changes have not been completely elucidated.
Objective: To measure serum markers of angiogenesis, inflammation and endothelial function in postmenopausal women screened for the METS.
Methods: Serum of 100 postmenopausal women was analyzed for angiopoietin-2, interleukin-8 (IL-8), soluble FAS ligand (sFASL), interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α), soluble CD40 ligand (sCD40L), plasminogen activator inhibitor-1 (PAI-1), and urokinase-type plasminogen activator (uPA). Comparisons were made in accordance to the presence or not of the METS and each of its components. Modified Adult Treatment Panel III criteria were used to define the METS.
Results: Women with the METS ($n = 57$) had similar age and time since menopause as compared to those without the syndrome ($n = 43$). In general, women with the METS displayed a trend for higher levels of the analyzed markers. Nevertheless, only IL-6 levels were found to be significantly higher and uPA levels significantly lower among METS women as compared to those without the syndrome. When analyte levels were compared as to presenting or not each of the diagnostic features of the METS, it was found that IL-6 levels were higher among women with abdominal obesity, low HDL-C and high triglyceride levels. Women with low HDL-C and high triglyceride levels presented significantly lower uPA levels and those with high glucose and low HDL-C displayed significantly higher sCD40L levels.
Conclusion: Postmenopausal women with the METS in this sample displayed higher IL-6 (inflammation) and lower uPA levels (endothelial dysfunction). These were mainly related to metabolic and lipid abnormalities. More research is warranted in this regard.

1. Introduction

The prevalence of the metabolic syndrome (METS) increases after the onset of the menopause. This syndrome is a cluster of variables closely related to obesity, inflammation, insulin resistance, prothrombosis and atherogenesis that increase cardiovascular disease, cancer and mortality risk [1–3]. It is a chronic condition related to lifestyle habits that affects a quarter of women and males worldwide [4]. The METS is associated with increased inflammation, endothelial dysfunction, oxidative stress and abnormalities in both the macro- and microvasculature [5]. Female menopausal transition and ageing affect metabolic and inflammatory pathways that relate to vascular dysfunction and increased metabolic and cardiovascular risk. The relative contribution to inflammation and vascular dysfunction related to the menopause or the METS remains to be elucidated. Hence, the aim of the present study was to measure serum markers of angiogenesis, inflammation and endothelial function in postmenopausal women with and without the METS and each of its components.

2. Methods

2.1. Participants and study design

A METS screening programme was carried out from December 2011 to June 2012 at the Institute of Biomedicine of the Medical

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Faculty of the *Universidad Católica de Santiago de Guayaquil*, Guayaquil, Ecuador [6]. A total of 204 natural postmenopausal women (40–65 years) participated in the programme recruited through newspaper advertising. All women were non-hormone therapy (HT) users. Those taking phytoestrogens or drugs intended to decrease lipid levels were excluded from the study. Research protocol of the study was reviewed and approved by the Scientific Research Committee of the Institute of Biomedicine. Eligible women were asked to attend the Institute to be informed about the study, its purposes and provide written consent of participation. Those consenting and fulfilling the inclusion criteria were asked to return after an 8 h overnight fast, moment in which sociodemographic data, waist circumference, weight, height and blood pressure measurements were recorded. In addition, a 10–15 ml peripheral venous blood sample was obtained.

To fulfil the aim of the present study, serum of 100 participants of the original cohort was reassessed and analyzed for angiopoietin-2, interleukin-8 (IL-8), and soluble FAS ligand (sFASL) (angiogenesis); interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) (inflammation); and soluble CD40 ligand (sCD40L), plasminogen activator inhibitor-1 (PAI-1) and urokinase-type plasminogen activator (uPA) (endothelial function). Analyte levels were compared in accordance to the presence or not of the METS and each of its components.

2.2. Diagnostic criteria for the metabolic syndrome

The METS was defined using Adult Treatment Panel III diagnostic criteria modified by the American Heart Association and the National Heart, Lung, and Blood Institute [7]. This was the case if three or more of five criteria were encountered: abdominal obesity (waist circumference >88 cm), increased serum triglycerides (TG) (≥150 mg/dL), decreased high density lipoprotein cholesterol (HDL-C) (<50 mg/dL), high fasting glucose (≥100 mg/dL, or the use of hypoglycemic agents) and increased blood pressure (≥130/85 mmHg, or the use of antihypertensive medications) [7]. Method for assessing abdominal perimeter has previously been described [6].

2.3. Serum assays

Blood samples taken from each participant were centrifuged at 5 °C for 10 min at 3000 rpm. The obtained serum was treated accordingly to manufacturer instructions, decanted into 0.5 ml aliquots and then stored at −70 °C. Subsequently, and before proceeding with analysis, DDP-IV inhibitor and aprotinin (Sigma–Aldrich, St. Louis, MO, USA) were added to the samples at a final concentration of 100 μM and 0.013%, respectively.

2.3.1. Measurement of the different analytes

TG, HDL-C and glucose levels were assayed using the enzymatic colorimetric method with a Hitachi 717 automatic photometric analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The concentration of angiopoietin-2, IL-8 and sFASL, IL-6, TNF-α, sCD40L, PAI-1, and uPA were measured using Bio-Plex 200 System® (Bio-Rad Laboratories, Inc., CA, USA) at the Bioclarma srl, Turin, Italy [8].

2.3.2. Assay format

The Bio-Plex® multiplex assay employs a standard enzyme immunoassay formatted on magnetic beads using a 96-well plate format. However, rather than a flat surface, it uses differentially detectable bead sets as a substrate reacting with analytes in solution. After a series of washes to remove unbound protein, a biotinylated detection antibody is added to create a sandwich complex. The final detection complex is formed with the addition of streptavidin–phycoerythrin conjugate. Phycoerythrin serves as a fluorescent indicator or reporter. The use of differentially detectable beads enables the simultaneous identification and quantification of many analytes in the same sample (2 μl of serum sample) [9].

Data acquisition and analysis from the reactions are performed using a Bio-Plex system or similar Luminex-based reader. When a multiplex assay suspension is drawn into the Bio-Plex 200 reader, a red (635 nm) laser illuminates the fluorescent dyes within each bead to provide bead classification and thus assay identification. At the same time, a green (532 nm) laser excites phycoerythin to generate a reporter signal, which is detected by a photomultiplier tube. A high-speed digital processor manages data output, and the Bio-Plex Manager™ software (version 6.1, Bioclarma Research and Molecular Diagnostics, Torino, Italy) presents data as median fluorescence intensity (MFI) as well as concentration (pg/ml). The concentration of the analyte bound to each bead is proportional to the MFI of reporter signal [10].

2.4. Statistical analysis

Statistical analysis was performed using the GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA) and the Statistical Package for the Social Sciences (IBM SPSS, Armonk, NY, USA). Data are presented as mean ± standard deviations, frequencies and percentages. The Mann–Whitney *U* test was used to analyze group comparison differences (continuous data) and the chi-square test to analyze percentages. Spearman coefficients were calculated to determine correlations between studied analyte levels and the components of the METS expressed as numeric variables. Additionally, multiple linear regression analysis was performed adjusting for several confounding factors (female age, time since menopause onset, BMI, parity). A p value of <0.05 was considered as significant.

3. Results

A 57% (n = 57/100) of the analyzed serum samples were defined as METS and 43% (n = 43) as non METS (controls). A 29% and 10% of women indicated being hypertense and diabetic, respectively. Age and time since menopause onset were similar in both studied groups. Women with the METS presented a higher rate of modified ATP III diagnostic criteria and a non-significant trend for higher levels of angiopoietin-2, IL-8 and sFASL (Table 1). These molecules are involved in processes of angiogenesis. Markers of general inflammation, such as TNF-α, and of endothelial dysfunction, such as PAI-1 and sCD40L, were not different amongst groups. However, the inflammatory cytokine IL-6 was significantly higher in women with the METS, and uPA, a marker of endothelial function associated with activation of fibrinolysis, was instead significantly lower in METS women (Table 1 and Fig. 1). When analyte levels were compared as to presenting or not each of the METS components it was found that IL-6 levels were higher among women with abdominal obesity, low HDL-C and high TG levels. Women with low HDL-C and high TG levels presented significantly lower uPA levels and those with high glucose and low HDL-C displayed significantly higher sCD40L levels (Data not shown on Table). Upon bivariate Spearman analysis, IL-6 levels positively correlated with abdominal perimeter and TG levels (r^2 = 0.40 and 0.38, respectively, both p = 0.02), and inversely with HDL-C (r^2 = −0.37, p = 0.01). uPA levels directly correlated with HDL-C levels and inversely with TG levels (r^2 = 0.39 and −0.36, respectively, both p = 0.01). There was a positive correlation between sCD40L and glucose levels (r^2 = 0.42 and 0.37, respectively, both p = 0.01), and an inverse correlation with HDL-C levels (r^2 = −0.36, p = 0.01). These correlations were confirmed after multiple linear regression analysis controlling for several confounding factors.