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Pro-inflammatory cytokine levels in postmenopausal women with the metabolic syndrome

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Abstract

Background. Prevalence of the metabolic syndrome (METS) and its components significantly increase after the menopause. Related increased cardiovascular risk may partially be explained by a pro-inflammatory state.

Objective. To assess pro-inflammatory cytokine serum levels in postmenopausal women with and without the METS.

Methods. Serum of 90 postmenopausal women who previously participated in a METS screening programme was analysed for tumour necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6). Cytokine levels were compared among those with and without the syndrome, and for each of its components. Linear relationships were also assessed between cytokine levels and several continuous variables including each diagnostic METS criteria and menopausal symptoms assessed with the Menopause Specific Quality of Life tool (MENQOL).

Results. For all studied women mean age was 55.1 ± 7.3 years with 63.3% having abdominal obesity, 15.6% hyperglycaemia, 58.9% high triglycerides, 44.4% hypertension and 25.6% high total cholesterol levels. Women with the METS ($n = 45$) significantly had higher body mass index values, and higher rates of abdominal obesity, hyperglycaemia, hypertriglyceridemia, hypertension and lower HDL-C levels. Cytokine levels did not differ among women with or without the METS; however, independent of METS diagnosis those with abdominal obesity displayed significantly higher IL-6 levels and those with hypertension higher levels of both cytokines. Levels of both cytokines positively correlated with age and time since menopause, IL-6 positively correlating with waist circumference values and TNF-α positively with both systolic and diastolic blood pressure levels. A significant positive correlation was also found between the number of positive METS criteria (0–5) and both cytokine levels. Cytokine levels did not correlate with vasomotor and psycho-social MENQOL scores.

Conclusion. Pro-inflammatory cytokine levels in this postmenopausal series positively correlated with age, time since the menopause, abdominal circumference, blood pressures levels and the number of positive METS diagnostic criteria. There is a need for more research in this regard.

Keywords: *Postmenopause, metabolic syndrome, cytokines, obesity, hypertension, interleukin 6, tumour necrosis factor α*

Introduction

Female cardiovascular risk is currently a major health concern. This risk increases with age and in relation to obesity, hypertension, insulin resistance and dyslipidemia, entities commonly grouped under the definition of the metabolic syndrome (METS) [1–3]. The vascular system receives many endocrine and metabolic influences [4–6]; hence, involved biochemical pathways in obesity, hypertension and the METS are complex and difficult to understand. The adipose tissue is an active secretory organ that produces a variety of molecules known as adipocytokines, including the tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), leptin, adiponectin and resistin, that mediate many metabolic changes in the METS. Obesity is frequently associated with insulin resistance and is the main metabolic alteration seen in the METS. Altered insulin function appears to result from a state of low-grade systemic inflammation [7,8]. In this sense, adipocytokines have an important role in the pathophysiology of the METS by acting on insulin signalling, fibrinolysis and endothelial cell adhesion [9–11].

After the menopause the prevalence of the METS increases. In Ecuador, it has been previously reported that 41.6% of postmenopausal women present this syndrome [12]. Several studies seem to point out to the fact that cytokine secretion among postmenopausal women with the METS may be altered [5,11,13,14]. Therefore, the aim of the present study was to assess pro-inflammatory cytokine serum levels in postmenopausal women with and without the METS.

Methods

Study design and subjects

From 1 February 2005 to 31 March 2005 a METS screening programme was carried out at the Institute of

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Biomedicine of the Medical Faculty of the Universidad Católica of Guayaquil Ecuador [12]. A total of 325 natural postmenopausal women (amenorrhea > 1 year), aged 40 or more, non-hormone therapy (HT) users participated. All subjects were originally informed about the research, its purposes and written consent obtained. Those consenting were asked to return after an 8 h overnight fast, when socio-demographic data, waist circumference and blood pressure measurements were recorded. After a 10 min resting period in sitting position, mean blood pressure was determined by performing two separate determinations 10 min apart. Waist circumference expressed in centimetres was obtained from women in supine position. Weight (kg) and height (m) were also recorded. Additionally a 10–15 ml peripheral venous blood sample was obtained for serum biochemical analysis. Definition of mestizo race, rural residency and low economic income within our population has been described in detail elsewhere [15]. Subjects were defined as sedentary if carrying out less than 15 min of physical activity twice per week [16]. Participants taking drugs intended to decrease lipid levels were excluded.

To fulfil the aims of the present study, serum of 45 women with METS randomly selected from the original cohort were reassessed and analysed for TNF-α and IL-6. For each case a control matched for age and time since the menopause was selected. Cytokine levels were compared between cases and controls and for each and the number of the METS components. Research protocol of this case control study and that of the original screening programme were approved by the Bioethics Committee of the Medical Faculty of the Universidad Católica de Santiago de Guayaquil, Ecuador.

Diagnostic criteria for the METS

Diagnostic criteria of the Third Expert Panel in Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (ATP III) [1] were used to define the METS. This was the case if three or more of five risk determinants were found: abdominal obesity (waist circumference > 88 cm), increased serum triglycerides (TG) ($\geq$150 mg/dl), decreased high density lipoprotein cholesterol (HDL-C) (< 50 mg/dl), high fasting glucose ($\geq$110 mg/dl) and increased blood pressure ($\geq$130/85 mmHg) [1]. Additionally, ATP III cut-off values set at 240 and 160 mg/dl were used to define high total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels, respectively [1]. Women taking oral hypoglycaemic or antihypertensive medication prescribed by a physician were considered as diabetic or hypertense independent of the serum or blood pressure findings. Body mass index (BMI) was also calculated for each subject as weight (in kg) divided by the square of height (in m). Obesity was defined as a BMI $\geq$ 30 kg/m^2 [17].

Serum assays

Blood samples withdrawn from each original participant were centrifuged at 5°C for 10 min at 3000 rpm. Obtained serum was decanted into 1.5 and 2.0 ml aliquots and stored at −70°C until analysis. TC, TG, LDL-C, HDL-C and glucose levels were assayed with a Hitachi 717 automatic photometric analyser (Roche Diagnostics GmbH, Mannheim, Germany). IL-6 and TNF-α were assayed using enzyme amplified chemiluminescence method with an Immulite 1000 (Siemens Medical Solutions Diagnostics, Los Angeles, USA). Analytic sensitivity was 1.6 and 2 pg/ml for IL-6 and TNF-α respectively.

The Menopause Specific Quality of Life tool (MENQOL)

Quality of life was also measured among participants of the original programme [18], using the Spanish version of the MENQOL tool [19]. The questionnaire is composed of 29 items grouped in four domains: vasomotor, psycho-social, physical and sexual. Each item can be checked as non-present or present. In the latter case, the item is graded according to its severity from 0 to 6. No total score is available, rather a mean score within each domain is generated according to each subject's response. As previously reported, abdominal obesity and hypertension were related to higher vasomotor and psycho-social domain scores [18]. Hence obtained scores for these domains (in selected cases and controls) were included for correlation calculations with cytokine levels.

Statistical analysis

Statistical analysis was performed using SPSS (Version 10.0 for Windows, SPSS, Chicago, IL) and EPI-INFO 2000 statistical packages (Centers for Disease Control and Prevention, Atlanta, GA, USA/World Health Organization, Geneva, Switzerland). Data are presented as mean $\pm$ standard deviations, percentages, medians and interquartile ranges. Normality of data distribution was determined with the Kolmogorov Smirnov's test. According to this, continuous data were compared with paired student's t test (parametric) or Wilcoxon's rank test (non parametric) for two related samples; the Mann–Whitney test (non-parametric) for two independent and the Kruskal–Wallis test (non parametric) for various independent samples. Chi-square or Fisher's exact test were used to compare percentages. Pearson's or Spearman's correlation coefficients were used to determine linear relationships among cytokine levels and several continuous variables including each diagnostic METS criteria and vasomotor and psycho-social MENQOL scores. A p value < 0.05 was considered as statistically significant.

Results

Baseline characteristics of studied women are depicted on Table I. For all studied women mean age was 55.1 $\pm$ 7.3 years with 46.7% being sedentary, 63.3% having abdominal obesity, 15.6% hyperglycaemia, 58.9% high triglycerides, 44.4% hypertension and 25.6% high TC levels. Women with the METS significantly had higher BMI values, and higher rates of abdominal obesity, hyperglycaemia, hypertriglyceridemia, hypertension and lower HDL-C levels. Cytokine levels did not differ among women with or without the METS (Table I).

Cytokine levels and METS prevalence according to each diagnostic component are depicted on Table II. Independent of having the METS, women with abdominal obesity displayed significantly higher IL-6 levels and those with hypertension higher levels of both cytokines. METS prevalence was significantly higher in women presenting positive diagnostic components.

Coefficient correlations for cytokine levels and various continuous variables are presented in Table III. Levels of

Table I. Basal characteristics of studied women.

Parameters	All, $n=90$	METS, $n=45$	Non METS, $n=45$	p value*
Age (years)	55.1 ± 7.3 [54,9]	55.1 ± 7.4 [54,9]	55.0 ± 7.4 [54,9]	0.90†
Time since menopause onset (years)	7.1 ± 6 [4.5, 8]	7.1 ± 6 [5,8]	7.1 ± 6.0 [4, 7.5]	0.07‡
Mestizo (%)	81 (90.0)	41 (91.1)	40 (88.9)	0.72§
Urban residency (%)	81 (90.0)	39 (86.7)	42 (93.3)	0.29§
Low income (%)	53 (58.9)	26 (57.8)	27 (60.0)	0.83§
Tobacco use (%)	4 (4.4)	2 (4.4)	2 (4.4)	0.99§
History of cardiovascular event	3 (3.3)	3 (6.7)	0 (0.0)	0.31¶
Number of family members (mean)	3.9 ± 1.4 [4,2]	3.9 ± 1.6 [4,2]	4.0 ± 1.5 [4,2]	0.71†
Sedentary (%)	42 (46.7)	21 (46.7)	21 (46.7)	0.99§
Mean BMI (kg/m^2)	29.5 ± 4.5 [29.5, 22.7]	31.0 ± 4.6 [31.9, 5.7]	28.0 ± 4.0 [27.7, 5.8]	0.001†
Abdominal obesity > 88 cm (%)	57 (63.3)	41 (91.1)	16 (35.6)	0.0001§
Glycemia ≥110 mg/dl (%)	14 (15.6)	14 (31.1)	0 (0.0)	0.0001¶
Triglycerides ≥150 mg/dl (%)	53 (58.9)	37 (82.2)	16 (35.6)	0.0001§
HDL-C < 50 mg/dl (%)	55 (61.1)	39 (86.7)	16 (35.6)	0.0001§
Blood pressure ≥130/85 mmHg (%)	40 (44.4)	27 (60.0)	13 (28.9)	0.003§
Total cholesterol ≥240 mg/dl (%)	23 (25.6)	13 (28.9)	10 (22.2)	0.46§
LDL-C ≥160 mg/dl (%)	10 (11.1)	6 (13.3)	4 (8.9)	0.52§
IL-6 (pg/ml)	3.4 ± 2.2 [2.4, 2.1]	3.8 ± 2.5 [3.0, 2.6]	3.0 ± 1.9 [2.0, 1.7]	0.06‡
TNF-α (pg/ml)	8.2 ± 3.0 [7.9, 3.0]	8.7 ± 3.9 [8.0, 3.0]	7.7 ± 1.9 [7.9, 2.7]	0.38‡

Data are presented as mean ± standard deviations, percentages (%) or [median, interquartile range]; *p value after comparing women with and without the METS obtained with †student's t test, ‡Wilcoxon's rank test, the §chi-square test or ¶Fisher's exact test. BMI, body mass index; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

Table II. Cytokine levels and METS prevalence according to the diagnostic component.

Parameters	IL-6 (pg/ml)	TNF-α (pg/ml)	METS (%)
Abdominal obesity > 88 cm;			
Yes, $n=57$	3.7 ± 2.3 [3.3, 2.4]	8.4 ± 3.3 [8.1, 3.1]	41 (71.9)
No, $n=33$	3.0 ± 2.2 [2,1]	7.9 ± 2.6 [7.5, 2.4]	4 (12.1)
	[$p=0.01$]*	[$p=0.40$]*	[$p=0.0001$]§
Glycemia ≥110 mg/dl;			
Yes, $n=14$	3.3 ± 2.8 [2, 1.6]	8.7 ± 4.5 [7.6, 3.8]	14 (100.0)
No, $n=76$	3.5 ± 2.1 [2.5, 2.3]	8.1 ± 2.8 [7.9, 3.0]	0 (0.0)
	[$p=0.44$]*	[$p=0.80$]*	[$p=0.001$]¶
Triglycerides ≥150 mg/dl;			
Yes, $n=53$	3.4 ± 2.0 [2.3, 2.0]	8.3 ± 3.0 [8.1, 3.2]	37 (69.8)
No $n=37$	3.5 ± 2.5 [2.4, 2.3]	8.0 ± 3.3 [7.5, 2.3]	8 (21.6)
	[$p=0.76$]*	[$p=0.31$]*	[$p=0.0001$]§
HDL-C < 50 mg/dL;			
Yes, $n=55$;	3.6 ± 2.4 [2.5, 2.4]	8.6 ± 3.4 [7.9, 3.0]	39 (70.9)
No, $n=35$	3.1 ± 2.0 [2.0, 1.9]	7.7 ± 2.4 [8.1, 2.7]	6 (17.1)
	[$p=0.28$]*	[$p=0.26$]*	[$p=0.0001$]§
Blood pressure ≥130/85 mmHg;			
Yes, $n=40$;	3.8 ± 2.2 [3.1, 2.6]	9.0 ± 3.9 [8.2, 2.7]	27 (67.5)
No, $n=50$	3.1 ± 2.3 [2.0, 1.6]	7.6 ± 2.0 [7.5, 3.0]	18 (36.0)
	[$p=0.01$]*	[$p=0.04$]*	[$p=0.002$]§
Number of METS diagnostic criteria;			
3 $n=26$,	3.6 ± 2.7 [2.5, 2.3]	8.6 ± 4.4 [7.6, 3.2]	–
4 $n=15$,	4.5 ± 2.3 [4.1, 4.4]	8.7 ± 1.7 [8.8, 2.8]	
5, $n=4$	2.9 ± 0.9 [2.9, 1.6]	10.0 ± 6.4 [8.6, 11.5]	
	[$p=0.24$]†	[$p=0.40$]†	

Data are presented as mean ± standard deviations, percentages (%) or [median, interquartile range]; p values in square brackets were calculated with the *Mann–Whitney test, the †Kruskal–Wallis test or the §chi-square or ¶Fisher's exact test.

both cytokines positively correlated with age and time since menopause. IL-6 levels positively correlated with waist circumference values and TNF-α positively with both systolic and diastolic blood pressure levels. There was a significant positive correlation between the number of positive METS criteria (0–5) and both cytokine levels. Cytokine levels did not correlate with vasomotor and psycho-social MENQOL scores.

Table III. Correlations between cytokine levels and various continuous parameters.

	IL-6 (pg/ml)	TNF-α (pg/ml)
Age (years)	0.24 (0.02)	0.24 (0.02)
Time since menopause (years)	0.28 (0.01)	0.22 (0.03)
Waist circumference (cm)	0.27 (0.02)	0.10 (0.40)
Body mass index (kg/m^2)	0.11 (0.51)	0.10 (0.40)
Glycemia (mg/dl)	−0.05 (0.67)	−0.03 (0.80)
Triglyceride (mg/dl)	−0.10 (0.40)	−0.02 (0.86)
HDL-C (mg/dl)	−0.20 (0.08)	−0.14 (0.19)
Systolic blood pressure (mmHg)	0.13 (0.22)	0.22 (0.04)
Diastolic blood pressure (mmHg)	0.10 (0.41)	0.19 (0.04)
Number of METS criteria (0–5)	0.27 (0.02)	0.22 (0.03)
Vasomotor scores	0.14 (0.19)	0.13 (0.22)
Psycho-social scores	0.14 (0.18)	0.10 (0.34)

p values are given in parenthesis.

Discussion

Components of the METS such as obesity, hypertension, hyperlipidemia and other metabolic alterations are a spectrum of phenotypes that can produce systemic inflammation and precede the development of insulin resistance, type 2 diabetes and cardiovascular disease [11,20,21]. Criteria for defining the METS have varied overtime. One such criterion is that proposed by the ATP III [1] which was used during the original METS screening programme and the present case–control secondary study. METS cases maintained a similar profile to that of our original cohort [12]; that is, displaying significantly lower HDL-C and higher glucose, triglyceride and blood pressure values as compared to their controls matched for age and time since menopause.

IL-6 and TNF-α are multifunctional cytokines linked to a number of disorders (arthritis, depression, scleroderma, lupus, etc.) [22]. These cytokines are considered major cardiovascular risk biomarkers and the main stimulant for hepatic production of C-reactive protein (CRP) [23]. Levels of these two cytokines are determined by genetic, metabolic and environmental factors. Although both increase with oestrogen deficiency, response is less intense when compared to host reaction to infection or tissue injury [24–26]. TNF-α stimulates endothelin-1 and angiotensinogen secretion [27,28] and its gene locus participates in hypertension mediated by insulin resistance [29]. In our series, levels of both cytokines did not differ when women with and without the METS were compared. This is in contradiction with what has been reported by others [2,30]. These discrepancies could be explained, at least in part, by the diversity of METS diagnostic criteria used from study to study; in fact, some women may have obesity and no other diagnostic feature of the METS and still display higher cytokine levels, and hence higher cardiovascular risk. Women with abdominal obesity of our series displayed significantly higher IL-6 levels and those with hypertension higher levels for both cytokines. Moreover IL-6 levels positively correlated with waist circumference values and TNF-α positively with both systolic and diastolic blood pressure levels. A significant positive correlation between the number of positive METS criteria (0–5) and both cytokine levels was also found. For an individual subject, increased cardiovascular risk varies according to which component of the syndrome is dominant. Obesity and hypertension were among the most prevalent clusters in the present series (Table I). Our findings therefore seem to support the fact that each METS component should be individually analysed (including cytokine assessment) when cardiovascular risk is to be assessed and lifestyle changes be implemented.

Findings of the present series are similar to those reported for older obese individuals with the METS, although in older subjects of one study these differences were also significantly different for TNF-α and plasminogen activator inhibitor-1 [14]. Weight reduction in obese women decreases insulin resistance and serum inflammatory marker levels, including IL-6 and CRP [31]. Although none of our postmenopausal women was on HT, its use in this population has the property of blunting serum IL-6 levels [32].

Our findings support that of others who have individually linked inflammation (cytokine secretion) and several METS components (i.e. hypertension, obesity) with negative cardiovascular and metabolic outcomes [33–35]. In a nested case–control study of the Women's Health Initiative (WHI) population, after adjusting for lipid and non-lipid risk factors, CRP and IL-6 levels were significantly associated to a two-fold increase in coronary heart disease events [36]. In another WHI nested case–control sub-analysis, baseline IL-6 and CRP levels were significantly higher among type 2 diabetes cases as compared to controls- and the relative risk for future diabetes mellitus among women in the highest versus lowest quartile was 7.5 fold and 15.7 for IL-6 and CRP, respectively. These values were significant after adjusting for BMI, history of family diabetes, smoking, exercise, and alcohol or HT use [37]. Our results, and those found in the literature, support the importance of abdominal obesity and cytokines as components of the METS. In fact, some authors have proposed including inflammatory marker alterations as a METS diagnostic criteria [2].

Although hypertension is a multifactorial disease [38,39], that includes genetic factors, evidence suggests that inflammatory cytokines levels correlate with blood pressure and are involved in the atherosclerosis process [6,40–42]. Our results are in accordance with other reports indicating that TNF-α serum levels are higher in hypertense women as compared normotense ones [43,44]. In addition, involvement of cytokine gene variants has been proposed in the genesis of hypertension [45]. Pro-inflammatory cytokines may activate the sympathetic nervous system, affect vascular function and the secretion of endothelial derived factors involved in blood pressure control such as endothelin and nitric oxide. Hypertension is independently associated to IL-6, TNF-α and CRP levels [46]. Increase in arterial blood pressure in response to acute psychological stress is significantly blunted in unrestrained male IL-6 knockout (KO) mice as compared to their wild (non-KO) counterparts [47]. In this model, the sympathetic and renin–angiotensin systems were activated to similar degrees during the peak blood pressure response in both mice types, suggesting that IL-6 could serve as a mediator or modulator of the pressor effects caused by other mechanisms. The sustained increase in IL-6 would contribute to the development of chronic hypertension and cardiovascular disease. On the other hand, a centrally acting sympatholytic agent (moxonidine) reduces blood pressure in hypertensive postmenopausal women by reducing inflammatory cytokine TNF-α [48].

In the present series, both cytokines displayed a positive significant correlation with age and time since menopause; IL-6 levels significantly correlating with waist circumference (abdominal obesity) and TNF-α with both systolic and diastolic blood pressure levels. Central obesity, low-grade inflammation and insulin resistance increase after

menopause onset [21]. Our findings support the fact that elevated body weight and hypertension in peri- and postmenopausal women may partly explain increases in inflammatory markers such as those studied in the present series; hence as proposed by others increasing cardiovascular disease risk. The endocrine control of the vascular function is complex with gender differences involved [49]. During the menopause ovarian hormone levels decrease drastically and under this scenario women lose oestrogen's antioxidative protective effects on both the vasoconstrictor and vasodilator systems.

Although cytokine levels significantly correlated with the number of METS components (Table III), predicting higher cytokine levels in relation to the number of positive diagnostic criteria may not be the case. Cytokines are the expression of derangements caused by obesity and hypertension. Current evidence does not support the value of the METS (present or not) in predicting cardiovascular events [50,51]. Moreover it would seem that METS individuals are at no higher risk for myocardial infarctions than those with diabetes or hypertension alone [50,51]. Our results support the importance of the presence of each component individually.

Frequency and intensity of hot-flushes have been associated to elevated body weight and cardiovascular risk [52–55]. Indeed, a longitudinal study (peri- to postmenopausal follow-up) found that hot flushes related to aortic and coronary artery calcifications [56]. It has been postulated that vascular function differs in relation to vasomotor hot flushes. Cytokine levels increase under experimentally simulated hot flushes [57]. Indeed, women with hot flushes, as compared to those without, display higher IL-8 levels [58]. Mid-aged women presenting psychological symptoms also display increased cytokine secretion, including TNF-α and IL-6 [59]. Despite this, the present series found no significant correlation between cytokine levels and MENQOL scores (vasomotor and psychosocial); despite the fact that as previously reported women with obesity and hypertension (found to have higher cytokine levels in the present series) were those more symptomatic. More research in this regard is required.

As for the limitations of this study one can mention its sample size (convenience sample). Despite this it is worthy to mention that METS cases were randomly selected and maintained a similar metabolic profile than those of the original cohort. In light of our findings, a new perspective can be outlined when it comes to assessing cardiovascular risk: one must not consider the METS as a whole yet assess each of its components individually, including cytokine measurement.

In conclusion, pro-inflammatory cytokine levels in this postmenopausal series positively correlated with age, time since the menopause, abdominal circumference, blood pressures levels and the number of positive METS diagnostic criteria. Our results seem to support the fact that each METS component should be individually recognised, and in this context cardiovascular risk assessed and lifestyle changes be implemented. There is a need for more research in this regard.

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