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Short Communication

Oxidative stress and menopause-related hot flashes may be independent events

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ABSTRACT

Objective: At present, there is growing demand for alternative, or additional, treatments to hormone replacement therapy for menopause-related hot flashes (HF). Antioxidant supplements have been recently proposed as possible candidates for this purpose, regardless of the absence of clear evidence in support of a link between these vasomotor symptoms and oxidative stress (OxS). The aim of our study was to evaluate the association between HF and OxS serum markers in a large sample of middle-aged women.

Materials and methods: We conducted a cross-sectional study on 245 perimenopausal and early postmenopausal women (age 45–60 years). The variables examined were presence of self-reported HF and levels of 8-iso-prostaglandin F2α, 8-OH-deoxy-2′-guanosine, advanced oxidation protein products, total antioxidant power, uric acid, thiols, and paroxonase-1.

Results: Seventy-six women (31%) reported to suffer from HF (either medium or high intensity). None of the peripheral markers of OxS examined was found to be significantly associated with the presence of HF.

Conclusion: Taken together, our data suggest that systemic OxS might not be implicated with the onset of the climacteric vasomotor symptoms that most commonly affect women experiencing perimenopause and early postmenopause.

Introduction

At present, the etiology of menopausal vasomotor symptoms (VS) is not fully understood. However, emerging evidence suggests that these disturbances may occur within a reduced thermoneutral zone caused by an ineffective interaction between norepinephrine and 17 β-estradiol (E2)-regulated α2-adrenergic receptors [1,2]. Several studies have also indicated that the occurrence of VS may represent not merely an effect of estrogen deficiency that can impair the quality of life, but also an early indicator of cardiovascular disease (CVD) risk [3,4]. Indeed, the presence and severity of night sweats, and, in particular, hot flashes (HF) is correlated with a proatherogenic condition featuring high blood pressure and cholesterol levels, as well as impaired endothelial function [3,4]. These findings have been interpreted by some authors as a clue of an implication of oxidative stress (OxS) in the etiology of these climacteric disturbances [5–7]. Indeed, it is now widely recognized that OxS plays a key role in all the stages of atherogenesis, from endothelial dysfunction to atheromatous plaque formation and rupture [8]. Furthermore, OxS is known as one of the most important contributors to the increased risk of CVD associated with menopausal transition [9]. Indeed, postmenopausal women are regarded as more vulnerable to OxS-related damage than women

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in reproductive age, not only because of their advanced age (aging is mutually associated with an increase in OxS) but also because of lower levels of E2, which is believed to act as an antioxidant [10–12].

On these bases, the aim of the present study was to investigate the association between HF and serum markers of both OxS-induced oxidative damage and antioxidant levels, in a sample of 245 healthy, nonobese women of perimenopausal and postmenopausal status.

Materials and methods

The present study included a subset of women enrolled in an observational research study by the Menopause and Osteoporosis Centre of the University of Ferrara (Ferrara, Italy), aimed at evaluating the physical and physiological correlates of OxS in women. These studies were undertaken within the framework of a protocol approved by the Medical School Ethics Committee, and in accordance with the Declaration of Helsinki (World Medical Association, http://www.wma.net), and the guidelines for Good Clinical Practice (European Medicines Agency, http://www.ema.europa.eu). Women were recruited upon signing an informed consent. Eligible participants were Caucasian women aged 45–60 years in perimenopause and early postmenopause (stage 1b or 2, assessed according to the Stages of Reproductive Aging Workshop bleeding criteria [13]). In particular, women in perimenopause experienced an increased and persistent variability of menstrual cycle length. Women reporting periods of amenorrhea longer than 11 months were classified as postmenopausal. Additionally, to confirm the assignment of menopausal status, we determined the blood levels of follicle-stimulating hormone and E2 for each woman, by using commercially available chemiluminescent microparticle immunoassays (Architect, Abbott Park, IL, USA), as previously described [14]. Exclusion criteria were factors known to influence OxS such as, the use of exogenous sexual hormones (including vaginal estrogens), dietary supplementation with nutritional antioxidants, vegetarian or vegan diet, and the diagnosis of a chronic disease (e.g., diabetes and hypertension).

Vasomotor symptoms

The presence of HF was assessed via a questionnaire at each visit and on the same day as the blood draw. At the beginning of the study, two questions were asked: (1) did the woman experience any HF in the 2 weeks prior to her visit? (2) If so, did she experience HF that can be classified as moderate to severe (i.e., having HF for more than 5 days/wk, with an average of more than 4 HF/d, and with at least six or more HF in 1 day)? [4]. Based on the responses to these questions, a dichotomous variable was created as follows:

- Absence of HF:
  - if the answer was “No” to question (1)
  - if the answer was “Yes” to question (1) but “No” to question (2)
- Presence of HF:
  - if the answer was “Yes” to both questions (1) and (2)

Women who were enrolled within the past two years of the study ($n = 85$) were additionally evaluated for VS using the Greene climacteric scale [15]. This scale is composed of 21 items that estimate VS, anxiety, depression, somatic symptoms, and sexuality. Each item is rated according to its severity on a 4-point scale: not at all (0), a little (1), quite a bit (2), and extremely (3). In this study, we only evaluated the two items (frequency of HF and sweating at night) that are used to calculate the subscore for VS (range 0–6).

Biochemical assays

Blood and urine were collected after overnight fast. Serum samples were obtained from blood by centrifugation (4650*g* for 20 minutes) and stored at −80°C until analysis.

The concentration of free 8-iso-prostaglandin F2α, (8-isoPGF2α) was assessed in urine by enzyme immunoassay (Cayman Chemical, Ann Arbor, MI, USA) following the manufacturer's protocol. Butylated hydroxytoluene was added to each aliquot (final concentration 0.005%) to prevent oxidation during processing.

Urine levels of 8-OH-deoxy-2′-guanosine (8-OH-dG) were measured by competitive immune-enzymatic assay (StressMarq Biosciences Inc., Victoria, BC, Canada). The concentrations of 8-isoPGF2α and 8-OH-dG concentration were normalized to urinary creatinine concentration, and expressed as ng/mg creatinine.

The serum concentration (expressed as μM) of advanced oxidation protein products (AOPP) was determined by spectrophotometric detection according to Capeillère-Blandin et al [16].

The collective contribution of nonenzymatic antioxidants (vitamins A, E, and C, uric acid, etc.) was measured by serum total antioxidant power (TAP) by ferric reduction/antioxidant power (FRAP) method [17] and the results of this colorimetric assay were expressed as FRAP units, where 1 FRAP unit corresponds to the reduction of 100μM of Fe^{3+} reduced to Fe^{2+} in 6 minutes.

The concentration of serum uric acid (μM) was determined by direct colorimetric enzymatic method in which uric acid was oxidized by uricase coupled with peroxidase [18].

Total concentration of serum thiols (μM) was determined by colorimetric DTNB-based assay as described by Hu [19]. The measurement of serum paraoxonase-1 (PON-1) basal activity was performed by using paraoxon as substrate as described elsewhere [20]. PON-1 basal activity was expressed as U/mL, where one unit is equivalent to 1 nmol of paraoxon hydrolysed/min.

Statistical analysis

SPSS 17.00 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Unpaired Student *t* test (for normal variables) and Mann–Whitney *U* test (for non-normal variables) were used to identify significant differences in the two sample groups considered. The Chi-square test was used to compare differences in categorical variables. Spearman correlation analysis was implemented for checking if ordinal variables (Greene climacteric subscale for VS) were related to continuous variables (levels of OxS markers). A two-tailed $p < 0.05$ was considered statistically significant.

Results

The main characteristics, as well as the mean serum levels of OxS markers, of the enrolled women subdivided according to presence ($n = 76$, 31%) or absence ($n = 169$, 69%) of HF are reported in Table 1. Based on these results, it is clear that the two sample subgroups are very similar in age, years since menopause, physical features, and the levels of sex hormones. Likewise, Mann–Whitney *U* test showed that none of the OxS markers examined was significantly different between the two given subgroups.

In line with the aforementioned results, there was no significant correlation between OxS markers and VS score obtained by the Greene climacteric VS subscale, in the subset of 85 women who were enrolled in the last two years of the study (Table 2).

Discussion

The assessment of OxS in living organisms represents a crucial analytical challenge, mainly because the direct determination of