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Inflammatory biomarkers and telomere length in women with polycystic ovary syndrome

Daiana Cristina Chielli Pedroso, M.Sc.,[a] Cristiana Libardi Miranda-Furtado, Ph.D.,[a] Gislaine Satyko Kogure, M.Sc.,[a] Juliana Meola, Ph.D.,[a] Maja Okuka, B.S.,[b] Celso Silva, M.D.,[c] Rodrigo T. Calado, M.D., Ph.D.,[d] Rui Alberto Ferriani, M.D., Ph.D.,[a] David L. Keefe, M.D., Ph.D.,[e] and Rosana Maria dos Reis, M.D., Ph.D.[a]

[a] Department of Obstetrics and Gynecology, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil; [b] Department of Obstetrics and Gynecology, University of South Florida College of Medicine, Tampa, Florida; [c] Center for Reproductive Medicine, Orlando, Florida; [d] Department of Internal Medicine, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil; and [e] Department of Obstetrics and Gynecology, New York University Langone Medical Center, New York, New York

Objective: To analyze whether leukocyte telomere length (LTL) is impaired in women with polycystic ovary syndrome (PCOS).
Design: Case–control study.
Setting: Hospital.
Patient(s): A total of 274 women, including 150 patients with PCOS and 124 controls.
Intervention(s): None.
Main Outcome Measure(s): Body mass index (BMI), waist circumference, systemic arterial pressure, lipid profile, E_2, LH, T, androstenedione, PRL, TSH, sex hormone–binding globulin, C-reactive protein (CRP), homocysteine, free androgen index, and the homeostatic model of insulin sensitivity (HOMA-IR) index were analyzed. The LTL evaluation was measured by quantitative polymerase chain reaction.
Result(s): The PCOS group had higher values for weight, BMI, waist circumference, systolic arterial pressure, triglycerides, LH, T, insulin, CRP, free androgen index, and HOMA-IR compared with the control group. Sex hormone–binding globulin and E_2 levels were lower in the PCOS group than in the control group. The LTL did not differ between groups. Age, BMI, and HOMA-IR had no significant effect on LTL. The inflammatory biomarkers CRP and homocysteine were negatively correlated with LTL in patients with PCOS.
Conclusion(s): Our results showed no differences in LTL between patients with PCOS and controls, but CRP and homocysteine biomarkers negatively correlated with LTL in the PCOS group. (Fertil Steril® 2014;■:■–■. ©2014 by American Society for Reproductive Medicine.)
Key Words: Polycystic ovary syndrome, telomeres, inflammatory biomarkers, hyperandrogenism, oxidative stress

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Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders and affects approximately 6%–14% of women of reproductive age in developing countries (1, 2). Hyperandrogenism is the main finding in the pathophysiology of the chronic anovulation that occurs in PCOS (3). Another feature of PCOS is the high incidence of insulin resistance (IR), which is detected in approximately 50%–90% of patients (4, 5) and has been highlighted in the pathophysiology of this syndrome (6). Metabolic disorders such as obesity, dyslipidemia, arterial hypertension, and abnormal glucose metabolism are commonly associated with PCOS, which becomes a complex disease and a matter of concern for women's health because these patients present with risk factors for type 2 diabetes mellitus and cardiovascular disease (CVD) (1, 7).

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Reprint requests: Rosana Maria dos Reis, M.D., Ph.D., Gynecology and Obstetrics, Ribeirao Preto Medical School, University of São Paulo, Av Bandeirantes 3900, Campus da Usp, Ribeirão Preto, São Paulo 14049-900, Brazil (E-mail: romareis@fmrp.usp.br).

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Polycystic ovary syndrome is also a proinflammatory state. A meta-analysis demonstrated that circulating C-reactive protein (CRP) is a reliable biomarker of low-grade chronic inflammation in women with PCOS (8). There is also an association between increased levels of inflammatory biomarkers and IR in the disorder (9, 10). In addition, IR and hyperinsulinemia may cause deleterious metabolic effects, including elevated serum homocysteine (11), which has been identified as an independent risk marker for CVD (12, 13). Recently it has been suggested that the inflammatory biomarkers may influence telomere biology (14–18), whereby elevated inflammatory activity may accelerate telomere shortening by inducing the release of reactive oxygen species that in turn damage telomeric DNA via oxidative stress (19).

Telomeres are composed of repetitive DNA sequences (5′-TTAGGG-3′ sequence in mammals), which wrap and protect the end of the chromosome. Telomeres are essential for proper chromosome alignment and spindle integrity, ensuring equal separation of chromosomes during meiotic cell division (20). The ends of the chromosome are replicated by the action of a reverse transcriptase (telomerase), which is an enzyme complex comprising two functional units: telomerase RNA, the component that provides the RNA template for the telomere synthesis, and telomerase reverse transcriptase, the protein that catalyzes the addition of telomeric repeats (21). Telomerase is not the only factor that determines telomere shortening; the telomere erosion rate can also be modulated by environmental factors in humans. Several studies have demonstrated a direct correlation between age and telomere shortening (22). In addition, obesity, tobacco smoking, and psychological stress can also accelerate telomere shortening (23). In contrast, sex hormones such as androgens and estrogens have been associated with decreased telomere erosion due to their action in influencing telomerase expression and/or activity (24–27).

Metabolic disorders, increased levels of inflammatory biomarkers, and hyperandrogenism frequently found in women with PCOS may influence telomere length (18, 23, 26–28). Thus, the aim of the present study was to examine whether leukocyte telomere length (LTL) is impaired in women with PCOS.

MATERIALS AND METHODS

This was a case–control study of women recruited consecutively from 2010 to 2013, which was informed through a consent form approved by the Research Ethics Committee of the Clinics Hospital (institutional review board), Ribeirao Preto Medical School, University of São Paulo, Brazil, under protocol number 3505/2009 and signed by all participants (or guardians) involved in the study.

We used a convenience sample with a total of 274 volunteers recruited; 150 women had PCOS, and 124 were healthy women who constituted the control group. The women were selected from the Gynecology Endocrinology, Infertility, and Contraception Outpatient Services of the Human Reproduction Division, Department of Gynecology and Obstetrics, Clinics Hospital, Ribeirao Preto Medical School, University of São Paulo, Brazil.

The inclusion criterion for participation in the study was age 13–45 years, regardless of race or social status. Patients with PCOS were diagnosed by criteria established by the Androgen Excess Society (2006), which included clinical and/or biochemical signs of hyperandrogenism with oligo/amenorrhea or polycystic ovaries (3). The presence of oligo/amenorrhea and clinical and/or laboratory hyperandrogenism, as well as a period of more than 2 years from menarche, was considered for the inclusion of adolescents (29). The control group included women with regular menstrual cycles of 24–38 days.

The exclusion criteria were tobacco smoking, hormonal medication, pregnancy or lactation, and any other endocrine abnormality that could interfere with the hypothalamic–pituitary axis, such as hyperprolactinemia, late-onset congenital adrenal hyperplasia, and Cushing's syndrome.

Body mass index (BMI), blood pressure, and waist circumference (WC) were assessed. Glucose levels were accessed by the glucose oxidase method. The concentrations of insulin, LH, E_2, TSH, PRL, sex hormone–binding globulin (SHBG), CRP, and homocysteine levels were assessed by chemiluminescence (IMMULITE 2000 Immunoassay System; Siemens). Testosterone and androstenedione (A) were measured by RIA (Immulite 1000, Siemens). Total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and triglycerides (TG) were assessed by enzymatic method, and low-density lipoprotein cholesterol (LDL) was calculated using the Friedewald formula: [LDL = TC − (HDL + TG/5)] (30).

The free androgen index (FAI) was determined using total T (nmol/L)/SHBG (nmol/L) × 100 (31), and IR was quantified using the homeostatic model assessment (HOMA-IR) [[(fasting glycemia in mg/dL × 0.05551) × fasting insulin in μU/mL]/22.5] (32). The metabolic syndrome was evaluated according to criteria of the National Cholesterol Education Program Adult Treatment Panel III (33), with modifications in the cutoff for fasting glucose as proposed by the American Heart Association and National Heart, Lung, and Blood Institute (34, 35).

Analysis of LTL in the Peripheral Blood

Genomic DNA from peripheral blood leukocytes of the women was isolated using QIAamp DNA Micro kits (Qiagen), according to the manufacturer's instructions. Deoxyribonucleic acid concentration and integrity were determined using a spectrophotometer, Nanodrop 2000c (Thermo Scientific). Leukocyte telomere length was measured by quantitative polymerase chain reaction, as described previously (36–38), using the following primer sequences: T-Fw, 5′CGGTTTGTTTGG GTTTGGGTTTGGGTTTGGGTTTGGGTT3′ and T-Rv, 5′GGCTT GCCTTACCCTTACCCTTACCCTTACCCTTACCCT3′; S-Fw, 5′C AGCAAGTGGGAAGGTGTAATCC3′ and S-Rv, 5′CCCATTCTA TCATCAACGGGTACAA3′.

Each sample was assayed in triplicate with the Rotor-Gene SYBR Green PCR Master Mix (Qiagen). Leukocyte telomere length was determined by calculating the telomere to single copy gene ratio (T/S ratio) using ΔCt [Ct(telomere)/Ct(single gene)]. The T/S ratio of each sample (x) was normalized relative to the mean T/S ratio of the reference sample [2 − (ΔctxΔCtr) = 2 − ΔΔCt], which was used to construct standard curves, both as a reference and as a validation sample.