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Original Article

Iron stores and obesity are negatively associated with ovarian volume and anti-Müllerian hormone levels in women with polycystic ovary syndrome

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ABSTRACT

Objective: Obesity and insulin resistance are associated with increased iron stores, but have conflicting effects on ovarian reserve in women with polycystic ovary syndrome (PCOS). Iron-catalyzed oxidative stress might be detrimental to ovarian tissue and granulosa cell function. In this study we determined the association between body iron stores, obesity, and ovarian reserve in women with PCOS.

Materials and Methods: One hundred and fifty-six women diagnosed with PCOS according to Rotterdam criteria and 30 normoweight healthy control women were enrolled in this cross-sectional study. Ovarian volume, total antral follicle count, and the anti-Müllerian hormone (AMH) level were measured as an indicator of ovarian reserve.

Results: Ferritin and transferrin-bound iron levels were significantly higher in women with PCOS than normoweight controls. Obese women with PCOS had higher ferritin levels ($p = 0.006$), but lower AMH levels ($p < 0.0001$) than nonobese women with PCOS. Using univariate analysis, the AMH level and mean ovarian volume were inversely related to the ferritin level, homeostasis model assessment of insulin resistance, and body mass index in women with PCOS. Body mass index and ferritin level remained significantly correlated with a lower AMH level and reduced ovarian volume, respectively, after considering other confounding variables.

Conclusion: An elevated ferritin level and obesity were negatively associated with ovarian volume and the AMH level, respectively, in women with PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age; PCOS is characterized by chronic anovulation, hyperandrogenism, and polycystic ovaries. Women with PCOS are known to have a higher prevalence of obesity [1,2], metabolism-related disorders [3,4], and insulin resistance, which have been proposed to correlate with oxidative stress caused by excess body iron stores [5,6].

The accumulated iron in mouse ovaries and boar testes has been shown to accelerate gonadal damage during the aging process due to increasing oxidative stress [7,8]. Serum ferritin levels in men and women with transfusion-dependent thalassemia major correlate with the presence of hypogonadism due to injuries, not only involving the hypothalamus and pituitary glands, but also the gonads [9–11]; however, the relationship between iron stores and gonadal function has never been investigated in humans without hemochromatosis.

Anti-Müllerian hormone (AMH) is a sensitive marker of ovarian reserve and granulosa cell function [12,13], and is a predictor of ovarian aging [9,14,15]. Although the reproductive lifespan of women with PCOS has been reported to be no different to [16] or longer than normo-ovulatory women [17], there is a more rapid

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decline in the AMH level in aging women with PCOS than in those without PCOS [17].

Ferritin is the cellular storage protein for iron and has also been the most commonly used indicator of body iron stores in epidemiologic studies [18,19]. Body iron stores and ferritin levels have been reported to be associated with type 2 diabetes, obesity, metabolic syndrome, and nonalcoholic steatohepatitis in the general population [20–23] and in women with PCOS [5,6]—a finding likely related to the tissue injury and organ failure caused by iron-promoted generation of reactive oxygen species.

Factors involved in the process of oxidative stress and the insulin-signaling pathway may be related to granulosa cell dysfunction [24,25]. In addition, obese and diabetic women tend to have an earlier decline of ovarian reserve and an earlier onset of the menopause [26,27]; however, the relationships between ovarian morphology, obesity, and metabolic disturbances in women with PCOS remain controversial [28,29].

We hypothesize that obesity and insulin resistance-related high ferritin levels in women with PCOS might be associated with decreased ovarian reserve due to potential oxidative injuries to the ovary. The aim of this study was to determine the relationships between obesity, ferritin level, and ovarian reserve in women with PCOS. The effects of oligomenorrhea and obesity on ferritin levels were also determined.

Material and Methods

Patients and data collection

One hundred and fifty-six women with PCOS and a chief complaint of irregular menstrual cycles and/or clinical hyperandrogenism, and willing to give informed consent, were consecutively recruited from the Reproductive Endocrinology Clinic (National Taiwan University Hospital, Taipei, Taiwan) at their first visit in this cross-sectional study. Thirty healthy normoweight volunteers with regular ovulatory cycles (mean cycle length < 35 days), < 30 years of age, and without signs of clinical or biochemical hyperandrogenism served as the control group. Control volunteers, but not PCOS patients, were enrolled by the aid of advertisement. None of the volunteers had been prescribed medications before enrollment. This study protocol was approved by the Research Ethics Committee of the National Taiwan University Hospital, Taipei, Taiwan. Written informed consent was obtained from all of the volunteers before participation.

The diagnosis of PCOS was based on the Rotterdam criteria, in which at least two of the following three criteria were met: (1) oligomenorrhea (< 8 spontaneous menstrual cycles per year at least 3 years before enrollment) or amenorrhea; (2) biochemical (serum total testosterone level $\geq$ 0.8 ng/mL) or phenotypic hyperandrogenism, including hirsutism and alopecia (acne was excluded due to an inconsistent correlation with hyperandrogenism in the literature); and (3) polycystic ovaries (> 12 follicles per ovary by transvaginal ultrasonography or an ovarian volume > 10 mL per ovary by transabdominal ultrasonography with a distended bladder for virginal women). The menstrual pattern data was obtained by questionnaire according to the patient's personal annotation or recall. The average of total spontaneous menstrual bleeding events every year for the continuous 3 years before enrollment was counted to define the severity of oligomenorrhea. Women with amenorrhea were defined as having no spontaneous menstrual bleeding without medication and receiving less than one induced menstrual bleeding cycle every year for at least 3 years before enrollment. All of the study volunteers were enrolled after excluding other endocrine, organic, and systemic abnormalities, such as hyperprolactinemia, thyroid dysfunction, Cushing's syndrome, congenital adrenal hyperplasia, adrenal tumors, ovarian tumors, autoimmune diseases, malignancies, central nervous system diseases, current use of oral contraceptives, or the use of medications known to affect the hypothalamic-pituitary-ovarian axis (antiandrogens, ovulation induction agents, antidiabetic medications, antiobesity medications, or glucocorticoids). None of the volunteers received a blood transfusion within 6 months before enrollment.

Overnight fasting blood samples were collected from PCOS patients with amenorrhea for > 3 months before hormone-induced withdrawal bleeding, and in the early follicular phase from those with PCOS who ovulated spontaneously. The blood sample was discarded if the serum progesterone level was > 2 ng/mL, or the serum estradiol level was > 150 pg/mL to exclude the possibility of delayed ovulation. The process for blood sample collection has been described in detail previously [4,30]. Blood was processed within 30 minutes of collection, and the blood glucose and insulin levels were determined on the day of sampling. The remaining serum and plasma were frozen at −70℃ until assayed.

Obesity was defined as a body mass index (BMI) $\geq$ 27 kg/m^2 based on the suggestion for the Asian as Hong Kong Chinese, Singaporean, and Indonesians [2].

Measurement of the total antral follicle count and mean ovarian volume

The mean ovarian volume was measured with transvaginal ultrasound for women with sexual experience and transabdominal ultrasound with a distended bladder for virginal women. The total antral follicle count (AFC) measurement was only performed for those who underwent transvaginal ultrasound examination ($n = 75$ in the PCOS group and $n = 12$ in the control group). Pelvic ultrasound measurements of the total AFC and mean ovarian volume were obtained according to a standard protocol [31] by one physician who was blind to the clinical manifestations of the enrolled volunteers. Briefly, after the longest medial axis of the ovary had been determined, the second dimension was measured, and then the probe was rotated 90° to obtain the third dimension. Ovarian volume was calculated using a suggested simplified formula (0.5 × length × width × thickness) [32]. The mean ovarian volume was defined as the average ovarian volume based on bilateral ovarian measurements. With respect to the AFC measurement, each ovary was scanned in longitudinal and transverse cross-sections from the inner to the outer margins to enumerate the follicles measuring between 2 mm and 9 mm in diameter. The total AFC was defined as the sum of the total number of follicles counted in both ovaries.

Assay methods

The levels of follicle stimulating hormone, luteinizing hormone (LH), estradiol, progesterone, total testosterone, sex hormone binding globulin, insulin, and glucose, and the homeostasis model assessment of insulin resistance (HOMA-IR) were measured and calculated as described previously [3]. The quantitative insulin sensitivity check index (QUICKI) was also applied to evaluate insulin resistance in women with PCOS. The serum levels of ferritin and transferrin-bound iron were measured with a biochemical autoanalyzer (TBA-2000FR; Toshiba Medical Systems Cooperation, Japan). Serum AMH levels were assessed, as in our previous studies [11,31], using a second-generation enzyme immunoassay (Immunotech A Bechman Coulter Company, Marseilles, France), according to the supplier's instructions. The free androgen index was used to estimate the bioavailable testosterone. The intra- and interassay coefficients of variation of all assays were < 10%.