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Müllerian-inhibiting substance in women with polycystic ovary syndrome: relationship with hormonal and metabolic characteristics

The aim of the present study was to examine the relationship between Müllerian-inhibiting substance (MIS) levels and metabolic characteristics in women with polycystic ovary syndrome. Increased ovarian MIS production may exert a paracrine negative control on follicle growth sufficiently to prevent selection of a dominant follicle. (Fertil Steril® 2004;82:970–2. ©2004 by American Society for Reproductive Medicine.)

Müllerian-inhibiting substance (MIS) is a member of the transforming growth factor-β superfamily of growth factors (1). Expression of MIS is limited to testicular Sertoli cells and ovarian granulosa cells. After birth, MIS levels in males are low, but rapidly rise to peak values by late infancy, then slowly decrease to the adult range at puberty (2, 3). In females, MIS is undetectable at birth, then increases to overlap with the male range at puberty (3). A relevant role for MIS in the regulation of follicle selection and maturation has been hypothesized (4–6).

Müllerian-inhibiting substance is detected in serum from women of reproductive age, and its levels vary slightly with the menstrual cycle, reaching the peak value in the late follicular phase (3, 7–8). It recently has been shown that MIS production is increased in women with polycystic ovary syndrome (PCOS) compared with in controls (9, 10), and this has been thought to be the result of aberrant activities of the granulosa cells in the polycystic ovaries (9).

A relevant percentage of women with PCOS exhibit insulin resistance and compensatory hyperinsulinemia (11). It has been proposed that hyperinsulinemia could adversely influence ovulatory function in women by acting at both pituitary and ovarian levels (12–14); indeed, it has been demonstrated that the higher is the insulinemia in women with PCOS, the lower is the probability of spontaneous ovulation (15). Therefore the objective of the study was to assess the potential association of MIS with body mass index (BMI), T, E_2 levels, insulin resistance, and ovulatory function in women with PCOS.

Fourteen women with PCOS were recruited. All were diagnosed with PCOS on the basis of oligomenorrhea (fewer than six menstrual periods in the previous year; n = 8) or amenorrhea (n = 6) and concurrent hyperandrogenemia. Five had a BMI of >27. The mean BMI was 25.1 kg/m^2. Ten patients had a Ferriman-Gallwey score of >7. None of the women had hypo- or hyperthyroidism, Cushing syndrome, adrenal hyperplasia, or hyperprolactinemia. All showed transvaginal ultrasonographic evidence of polycystic ovaries. Ultrasonographic diagnosis of polycystic ovaries was based on the presence of 10 or more follicles (2–10 mm in diameter) in both ovaries. No patient was taking drugs or had any significant pathology.

Fifteen healthy women with normal menstrual cycles and normal ovarian appearance (as assessed by ultrasound scan) were selected as the control group. Mean age and BMI were 23 y and 24.6, respectively. Written informed consent was obtained from each subject.

Subjects entered the study during the follicular phase of a spontaneous or induced menstrual cycle (i.e., plasma level of progesterone <1.8 ng/mL). Height, weight, waist to hip ratio, and BMI were measured for each woman. Basal blood samples for LH, FSH, E_2, T, free T, sex hormone–binding globulin (SHBG), fasting glucose, insulin, and MIS were obtained at 8 AM after overnight bed rest and fasting.

Fasting glucose and insulin levels were determined and Homeostatic Model Assessment (HOMA) calculated (16). The HOMA-derived insulin resistance index was calculated by using the following formula: IR = [fasting insulin (μU/mL) × fasting glucose (mmol/L)]/22.5. The study was approved by the institutional review board of the University of Siena.

Plasma FSH, LH, SHBG, E_2, T, and free T (FT) levels were assayed by double-antibody RIA using commercial kits from Radim (Rome, Italy) for FSH and LH, from Sorin (Saluggia-VC, Italy) for T and E_2, from DPC (Los Angeles, CA) for FT, and from DSL Wherter (TX) for SHBG. Samples were assayed in duplicate at two dilutions. Samples from a given subject were analyzed for each hormone in the same assay to avoid interassay variation. Quality control pools at low, normal, and high LH, FSH, SHBG, E_2, T, and free

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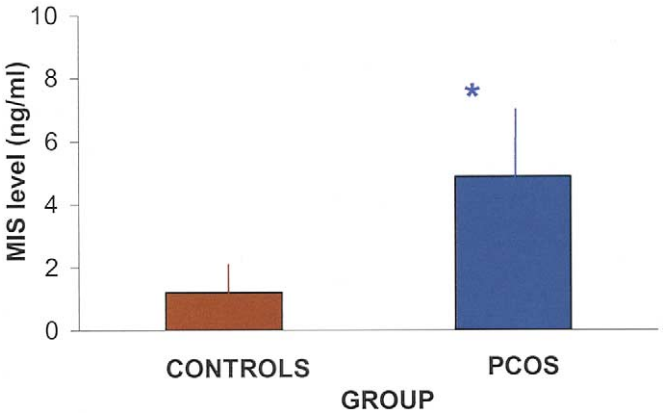
FIGURE 1

Müllerian-inhibiting substance levels were significantly higher in PCOS than in controls. *$P<.05$.



La Marea. Müllerian-inhibiting substance and PCOS. Fertil Steril 2004.

T concentrations were present in each assay. The detection limit of the assay was 0.20 IU/L for LH, 0.18 IU/L for FSH, 5 pg/mL for E_2, 80 pg/mL for T, 0.15 pg/mL for free T, and 2.5 nmol/L for SHBG 277. Intra- and interassay variations were 7.8% and 8.2% for LH; 6.2% and 6.5% for FSH; 4.2% and 4.9% for E_2; 3.4% and 4.6% for T, 3.2% and 3.4% for FT, 5.6% and 4.6% for SHBG.

Serum MIS was measured with the MIS ELISA kit (Immunotech, Marseilles, France). Briefly, 25 μL of each serum sample was incubated in duplicate on a polystyrene plaque precoated with a monoclonal anti-MIS antibody. After a 1-h incubation, a second monoclonal anti-MIS antibody, coupled to biotin, was added, together with a streptavidin-horseradish peroxidase complex. After addition of TMB substrate, the resulting color reaction was quantified using a spectrophotometer at 450 nm. A preparation of purified recombinant human MIS was used to construct a standard curve. The limit of sensitivity of the assay was 0.7 pmol/L (0.1 ng/mL); inter- and intraassay coefficients of variation were 8.7% and 5.3%, respectively, for a serum MIS concentration of 35 pmol/L and were respectively 7.8% and 4.9% for a serum MIS concentration of 1,100 pmol/L. No cross-reaction was observed with pure transforming growth factor-β.

Results are expressed as means and SD. Comparisons between the two groups were calculated by unpaired *t* test if the data were normally distributed and anyway by Mann-Whitney because of the small size of the groups. Correlations between different parameters were determined by using bivariate correlation statistics and are expressed as Spearman correlation coefficients. Statistical analysis was performed by the software Statsoft. Statistical significance was set at $P<.05$.

Müllerian-inhibiting substance was detectable in all patients and controls and ranged from 1.5 to 13.8 ng/mL. Levels of MIS were significantly higher in women with PCOS than in controls (5 ± 1.8 vs. 1.3 ± 0.5 ng/mL; $P<.05$; Fig. 1). A significant positive correlation was found between MIS and HOMA index ($r = 0.621$; $P<.05$). Negative correlations were found between MIS and E_2 levels ($r = -0.3$; $P<.05$) and SHBG levels ($r = -0.5$; $P<.05$). The strongest correlation was between MIS and HOMA. Estradiol levels and SHBG levels were significantly correlated with HOMA score by univariate linear regression analysis, suggesting that E_2 and SHBG levels correlate with MIS because of their correlation with HOMA. No significant correlations were found between MIS and T or androstenedione.

Women with PCOS were divided in amenorrheic (less than two uterine bleedings for the year, n = 6) and oligomenorrhoic patients (more than two but less than six uterine bleedings for the year, n = 8). No significant differences were observed between amenorrheic and oligomenorrheic women in age, BMI, gonadotropins, sex steroids, and SHBG plasma levels, and HOMA score. However, it has been found that MIS levels were significantly higher in patients with amenorrhea than with oligomenorrhea (6 ± 2 vs. 3.5 ± 0.8 ng/mL; $P<.05$).

Results of the present study confirm previous observations of higher levels of MIS in serum from patients with PCOS (9, 10). Significant positive and negative correlations have been found between MIS and insulin resistance as calculated by HOMA formula and by MIS, E_2, and SHBG levels, respectively.

Müllerian-inhibiting substance is a homodimeric disulfide–linked glycoprotein that is strongly expressed in Sertoli cell from testicular differentiation up to puberty and, to a much lesser degree, in granulosa cells from birth up to menopause. In the adult rat ovary, MIS can be detected in the growing follicles but disappears before ovulation (17). In cultured rat granulosa cells, exogenous MIS inhibits aromatase synthesis (18) and inhibits the proliferation of granulosa luteal cells (19, 20). Müllerian-inhibiting substance and its receptor mRNA are highly expressed in granulosa cells of mainly preantral and small antral follicles (4, 21). Hence a relevant role for MIS in the regulation of follicle selection or maturation has been hypothesized (4). It has been demonstrated recently that MIS-null mice exhibit a faster decrease in the pool of primordial follicles (5). Alternatively, overexpression of MIS in mice resulted in retardation in meiosis and folliculogenesis (6), indicating an inhibitory effect of MIS on recruitment of primordial follicles into the pool of growing follicles.

In the present study, we observed higher serum MIS levels in women with PCOS than in controls, confirming results of previous studies (9, 10). The significant negative correlation between MIS and E_2 could be secondary to the inhibiting effect of MIS on aromatase function (18). Furthermore, we found higher MIS levels in amenorrheic than in oligomenorrheic women with PCOS, which could indicate a role for MIS in the pathogenesis of PCOS-related anovulation. Alternatively, high MIS values could reflect a more evident impairment in follicular development and granulosa cell function in the ovary of amenorrheic than oligomenorrheic PCOS women.

Women with PCOS are more insulin resistant and hyperinsulinemic than are age- and weight-matched normal women. Recently, simple methods have been elaborated for assessing insulin sensitivity while avoiding complex procedures, such as HOMA (16). The HOMA focuses on basal fasting insulin and glucose levels. The correlation between HOMA and clamp-derived insulin sensitivity is surprisingly good considering the simplicity of the formula (22).

Hyperinsulinemia turns out to have an increasingly central role in the complex pathogenesis of PCOS. Indeed, a significant correlation has been found between insulin resistance and abnormalities in ovarian function, and it has been proposed that the higher is the insulin resistance in PCOS patients, the lower is the probability of spontaneous ovulation (15). This implies that moderate or severe insulin resistance is associated with severe oligomenorrhea or amenorrhea.

A significant positive correlation has been found between HOMA score and serum MIS levels. At the moment, no data are available about a possible relationship between insulin and MIS synthesis and secretion. However, the correlation between MIS and insulin could be secondary to their relation with PCOS-related anovulation.

Mechanisms of anovulation in PCOS are not fully understood. Increased ovarian MIS production may exert a paracrine negative control on follicle growth and have a role on PCOS-related anovulation.

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