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LETTER TO THE EDITOR

Anti-Müllerian hormone as marker of ovarian reserve in patients with long-standing type 1 diabetes

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To the Editor,

Type 1 diabetes (T1D) is a chronic disease caused by ß-cell destruction, which leads to insulin deficiency. T1D can develop serious complications, as well as other autoimmune and gynaecological disorders (1, 2). An increased risk of infertility, mainly related to uncontrolled hyperglycaemia, has been postulated in women with T1D compared to women without diabetes, even independently of menstrual irregularities. Specifically, in animal models of T1D, uncontrolled diabetes led to reduced circulating levels of gonadotropins and sex hormones, as well as impaired LH pulsatility (3). Similarly, hypogonadotropic hypogonadism was observed even in clinical studies on women with T1D. Furthermore, several ovarian abnormalities, such as reduced survival of follicular and granulosa cells, alterations in oocyte maturation and follicular development, and impairment of ovarian steroidogenesis have been described in animal models of T1D (4). Besides hyperglycaemia, exogenous hyperinsulinemia that might ensue from chronic administration of insulin is believed to play a role in T1D ovarian dysfunction, possibly acting as a gonadotropin-like hormone, stimulating the recruitment and growth of large follicles (5). Furthermore, endothelial dysfunction might accelerate ovarian ageing, conceivably leading to a sharp decrease in ovarian reserve.

Endometriosis is an inflammatory disease and infertility is a major concern related to this condition (6). There is consistent evidence about the impaired ovarian reserve in patients with endometriosis, compared to healthy women.

Anti-Müllerian hormone (AMH) is a glycoprotein belonging to the transforming growth factor-ß family and is secreted by small ovarian follicles. As AMH serum levels strongly correlate with the antral follicle count (AFC), this hormone has emerged as a biomarker of ovarian reserve and reproductive function, with diagnostic and prognostic value in clinical practice (7).

The aim of this cross-sectional study was to comparatively evaluate AMH serum levels and AFC in long-standing T1D patients, in patients with endometriosis-related infertility and in healthy control women, to ascertain the effects of a long duration of T1D on ovarian reserve.

MATERIALS AND METHODS

One-hundred-forty-five (n.145) women of reproductive age, namely n.49 with long-standing T1D in

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basal-bolus treatment (mean age 36.0±4.0 years; diabetes duration 13.4±5.0 years), n.50 with endometriosis-related infertility (mean age 32.0±5.0 years) and n.46 healthy control subjects (mean age 28.6±6.0 years), were enrolled in the outpatient clinics of Policlinico Umberto I, "Sapienza" University Hospital of Rome. T1D was diagnosed according to the American Diabetes Association criteria (8). Diagnosis of endometriosis was performed in accordance with current recommendations (9). The exclusion criteria were as follows: recent diagnosis of T1D (diabetes duration <5 years); current use of estro-progestinic contraceptives; menstrual cycle alterations; previous or current malignancies, chemotherapy or radiotherapy; history of surgery of the pelvic region; any other active diseases or chronic medications. The protocol was approved by the hospital ethics committee and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants. A detailed medical history was recorded and the relevant anthropometric parameters were assessed (height, weight, body mass index [BMI]). A venous peripheral blood sample in the early follicular phase of the menstrual cycle (within the third day from the beginning of menstruation) was collected to assess AMH levels. All subjects, at enrolment, underwent a transvaginal ultrasound scan (Samsung Electronics, Seoul, South Korea), performed independently by two experienced gynaecologists, to determine the number of antral follicles. AFC was defined as the number of follicles between 2 and 10 mm in diameter, observed on each side.

AMH assay

Blood collection was performed following a standard protocol. Samples were collected in a red top Vacutainer (Becton, Dickinson and Company, Plymouth, UK), clotted 60–90 min and centrifuged for 10 min at 1300 x g at room temperature. The obtained serum fractions were subsequently aliquoted in 1.5 mL Eppendorf tubes (Eppendorf srl, Milano, Italy) and frozen at -80°C until testing.

AMH serum values were measured by ELISA method (Ansh Labs Ultra-Sensitive AMH/MIS ELISA, Webster, USA), a quantitative solid-phase three-step sandwich type non-competitive immunoassay, including two anti-AMH monoclonal antibodies specific for different epitopes of the AMH. The first monoclonal antibody was coated into microtiter wells for capturing the antigen with the other biotinylated antibody; a streptavidin horseradish peroxidase conjugate (SHRP) was utilized to bind the biotinylated antibody complex. The antibody-antigen biotin conjugate-SHRP complex bound to the well was detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate was determined spectrophotometrically by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as a reference filter. The absorbance measured was directly proportional to the concentration of AMH/MIS in the samples and calibrators. The analytical sensitivity of AMH assay was 0.02 ng/mL. The specificity was 95%. The intra-assay coefficient of variation was 5.6%. According to manufacturer's instructions, the value of 0.9 ng/mL was considered as the AMH cut-off to identify reduced ovarian reserve.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD). Categorical data are expressed as percentages. Continuous variables were tested for normality with Kolmogorov-Smirnov test. Differences between groups were tested with the one-way analysis of variance (ANOVA), for normally distributed continuous variables, or with the Kruskal-Wallis H test, for not normally distributed variables. As appropriate, a post hoc test was subsequently performed on each group pair. Categorical variables between groups were compared by Fisher's exact test. A p-value <0.05 was considered statistically significant. Statistical analysis was performed with IBM SPSS Statistics software version 23 (Chicago, IL, USA).

RESULTS

The clinical characteristics of the enrolled population are shown in Table I. Low age-specific serum levels of AMH, indicating a reduced ovarian reserve, were observed in 12% of long-standing T1D patients, 10% of endometriosis infertile patients and 4% of healthy women. The prevalence of low circulating AMH levels was very similar between

Table I. *Clinical characteristics of the enrolled population*

	T1D N.49	Endometriosis N.50	Healthy women N.46
Age (years)	36.0±4.0	32.0±5.0	28.6±6.0
BMI (Kg/m²)	26.8±4.0	25.7±4.0	26.1±5.0
Diabetes duration (years)	13.4±5.0	-	-

T1D and endometriosis infertile patients (12% *vs* 10%, p>0.05, respectively), whereas the prevalence of reduced ovarian reserve was significantly higher in T1D group compared to control group (12% *vs* 4%, p<0.05, respectively) and in endometriosis infertile group compared to control group (10% *vs* 4% p<0.02, respectively) (Fig. 1). AFC did not significantly differ between the study groups.

DISCUSSION

In this study, low serum concentration of AMH was a frequent finding in T1D patients with a long course of disease and the prevalence of this phenomenon was significantly higher in long-standing T1D with respect to healthy subjects. This result might mirror a reduced ovarian follicular pool in T1D women of reproductive age compared to age- and BMI-matched control subjects. Kadiroğulları et al. reported similar findings (10). However, T1D patients were younger and had slightly shorter diabetes duration. Soto et al. showed that AMH levels were significantly lower in T1D patients compared to control subjects of more advanced age, suggesting an earlier decline in ovarian follicle pool in T1D women during the fourth decade of life (11). To date, studies evaluating ovarian reserve in T1D patients between the third and the fourth decades of life are limited, despite the markedly increased risk of infertility reported in this age range. Kim et al. observed a significant association between T1D and lower AMH values. However, AMH concentrations in women with T1D compared to women without diabetes were significantly lower only in the subgroup of those under than 35 years of age, not in older women (12).

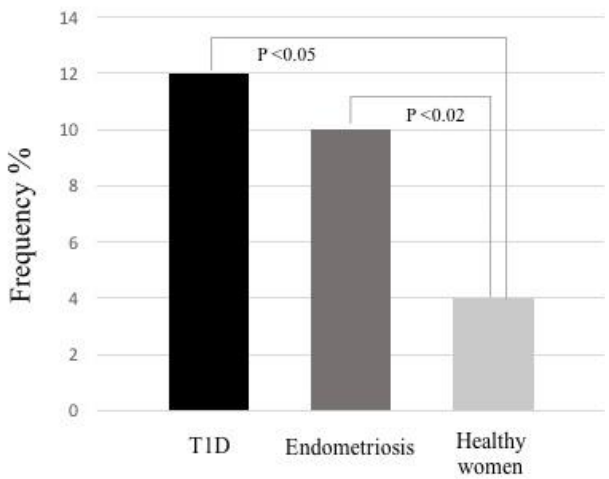

Fig. 1. *Prevalence of low age-specific AMH in the study population*

To our knowledge, this is the first study comparing ovarian reserve between patients with T1D patients and women affected by endometriosis. Remarkably, the proportion of patients with values of AMH under the cut-off to identify reduced ovarian reserve was comparable between these two groups. This finding suggests that T1D and endometriosis might similarly impair ovarian follicular pool and disturb ovarian function. Even though T1D and endometriosis are entirely different conditions, they might share several pathophysiological features, such as chronic inflammation and altered immune modulation, which have been hypothesized to be involved in the development of follicular damage and dysfunction.

In conclusion, alterations of AMH were common in T1D patients. Although the role of this marker should be further evaluated, the determination of serum AMH might be a useful clinical tool in pregnancy planning and fertility management in patients with T1D.

REFERENCES

1. Anastasi E, Filardi T, Tartaglione S, Lenzi A, Angeloni A, Morano S. Linking type 2 diabetes and gynecological cancer: an introductory overview. Clin Chem Lab Med 2018; 56(9):1413-25.
2. Tiberti C, Panimolle F, Bonamico M, et al. Long-standing type 1 diabetes: patients with adult-onset develop celiac-specific immunoreactivity more frequently than patients with childhood-onset diabetes, in a disease duration-dependent manner. Acta Diabetol 2014; 51(4):675-78.
3. Katayama S, Brownscheidle CM, Wootten V, Lee JB, Shimaoka K. Absent or delayed preovulatory luteinizing hormone surge in experimental diabetes mellitus. Diabetes 1984; 33(4):324-27.
4. Chang AS, Dale AN, Moley KH. Maternal diabetes adversely affects preovulatory oocyte maturation, development, and granulosa cell apoptosis. Endocrinology 2005; 146(5):2445-53.
5. Nandi A, Poretsky L. Diabetes and the female reproductive system. Endocrinol Metab Clin North Am 2013; 42(4):915-46.
6. Anastasi E, Fuggetta E, De Vito C, et al. Low levels of 25-OH vitamin D in women with endometriosis and associated pelvic pain. Clin Chem Lab Med 2017; 55(12):e282-e284.
7. Tremellen KP, Kolo M, Gilmore A, Lekamge DN. Anti-mullerian hormone as a marker of ovarian reserve. Aust N Z J Obstet Gynaecol 2005; 45(1):20-24.
8. American Diabetes Association. Standards of medical care in diabetes - 2020. https://care.diabetesjournals.org/content/43/Supplement_1. Accessed 8th June 2020.
9. Dunselman GA, Vermeulen N, Becker C, et al. ESHRE guideline: management of women with endometriosis. Hum Reprod 2014; 29(3):400-12.
10. Kadirogullari P, Demir E, Bahat PY, Kiyak H, Seckin KD. Evaluation of relationship between HbA1c levels and ovarian reserve in patients with type 1 diabetes mellitus. Gynecol Endocrinol 2020; 36(5):426-30.
11. Soto N, Iniguez G, Lopez P, Larenas G, Mujica V, Rey RA, Codner E. Anti-Mullerian hormone and inhibin B levels as markers of premature ovarian aging and transition to menopause in type 1 diabetes mellitus. Hum Reprod 2009; 24(11):2838-44.
12. Kim C, Karvonen-Gutierrez C, Kong S, et al. Antimullerian hormone among women with and without type 1 diabetes: the Epidemiology of Diabetes Interventions and Complications Study and the Michigan Bone Health and Metabolism Study. Fertil Steril 2016; 106(6):1446-52.