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AMH IN AUTOIMMUNE THYROIDITIS

Anti-Müllerian hormone as a marker of premature ovarian aging in autoimmune thyroid disease

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Abstract

There is an increased incidence of autoimmune thyroid disease (AITD) in women with infertility. We hypothesized that serum anti-Müllerian hormone (AMH) levels will be lower in premenopausal women with AITD than controls. We evaluated ovarian reserve in women with AITD ($n = 85$) and healthy controls ($n = 80$), all <40 years old. Detailed data on reproductive history were obtained. Gonadotrophins, steroids, AMH, and inhibin B levels were measured during the follicular phase. The number of pregnancies as well as live births was lower in women with AITD ($p < 0.01$). No difference was observed in terms of FSH, estradiol, and inhibin B. AMH levels were lower in AITD women than in controls (1.16 + 0.17 versus 1.28 + 0.25 ng/ml, mean + SD, $p = 0.001$). According to the multiple regression analysis, even after age adjustment, AITD was significantly and independently affected AMH levels ($t = 2.674$, $p = 0.008$). Women with AITD seem to have a diminished ovarian follicular reserve and measurement of serum AMH level has the potential to be used to predict this comorbidity.

Keywords

Folliculogenesis, infertilitiy, ovary, thyroid

History

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Introduction

Autoimmune thyroid disease (AITD) is the most common endocrine disorder in women of reproductive age. Its prevalence varies between 5% and 15% but the disease may often remain undiagnosed for several years because it may be present without overt thyroid dysfunction [1,2]. Various studies have focused on the association of AITD with reproductive failure and the overall conclusion favors a significantly increased incidence of AITD in women with infertility although the underlying pathogenic mechanisms remain largely speculative [3–9].

Anti-Müllerian hormone (AMH), also known as the Müllerian inhibiting substance, is a dimeric glycoprotein exclusively produced by granulosa cells of preantral (primary and secondary) and small antral follicles [10]. The number of small antral follicles is directly related to the total size of the primordial follicle pool [11]. Measurable quantities of AMH appear in serum and with the decrease in the number of antral follicles with age, AMH serum levels also become diminished [12]. AMH serum levels represent ovarian quantitative reserve in *in vitro* fertilisation (IVF) patients and may provide an index of age at menopause [13,14].

Inhibin B is another hormone which is primarily produced by the granulosa cells in follicle stimulating hormone (FSH) sensitive cohort of antral follicles. A decrease in inhibin B secretion has been associated with decreased oocyte quality and fertility potential [15]. Additionally, elevated levels have been reported in autoimmune oophoritis [16].

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In this study, we determined serum levels of AMH, inhibin B, steroids, and gonadotrophins in women with AITD to evaluate the ovarian reserve in this group of patients. We also aimed to test the hypothesis that serum AMH levels will be lower in premenopausal women with AITD compared with an age-matched group of control women.

Methods

Women with AITD ($n = 85$) were consecutively recruited from endocrinology outpatient clinics into this study between 2012 and 2013. Patients fulfilled the following inclusion criteria: positivity for one or more of the serum thyroid antibodies, euthyroidism, and ultrasonography (USG) findings consistent with thyroiditis. Patients and healthy control women ($n = 82$) were younger than 40 years, and the two groups were matched according to the gynecological and chronological age and body mass index (BMI). Gynecological age was calculated as chronological age minus the age of menarche. Healthy controls were chosen from among the subjects attending outpatient clinics for check-up. Women in the control group had regular menstrual cycles, normal thyroid function, and normal thyroid gland at USG. All control subjects denied having any comorbid condition including thyroid diseases.

The exclusion criteria were abnormal thyroid function tests, history of a previous thyroid dysfunction (subclinical thyroid dysfunction, overt hypothyroidism and hyperthyroidism, thyroid replacement, thyroid surgery, and radioactive iodine treatment), pregnancy, use of sex steroids, and any drug known to affect thyroid or ovarian function during the last 1 year, chronic non-thyroidal illnesses including Cushing's syndrome, celiac disease, genetic syndromes, renal disease, liver disease, cardiac disease, and undernourishment. The study protocol was approved by the local ethics committee and all the patients and control subjects gave written informed consent.

Euthyroidism was defined as thyroid stimulating hormone (TSH), free tri-iodothyronine (fT3), and free thyroxine (fT4) within the normal reference range. Serum fT4, fT3, TSH, antithyroglobulin (anti-Tg), and antithyroperoxidase (anti-TPO) levels had been measured by immunoassay (Immulite 2000, DPC Diagnostics, Los Angeles, CA and UniCel DxI 800, Beckman Coulter, CA) using commercial kits in venous blood samples obtained between 8:00 and 9:00 h while fasting. The thyroid USG was performed using Esaote Colour Doppler US (MAG Technology Co, Ltd. Model: 796FDII Yung-ho City, Taipei; Taiwan) and a 5.5–12.5 MHz linear array transducer by an experienced endocrinologist. The presence of a diffuse hypoechoic or heterogenous echotexture with or without hyperechoic lines was considered suggestive of thyroiditis.

An early morning sample of blood was obtained from both groups of women during the follicular phase (days 3–5). This blood was used for the measurement of AMH, inhibin B, gonadotrophins, estradiol, testosterone, 17OH progesterone, and dehydroepiandrosterone sulfate. Steroids and gonadotrophins were measured as described previously [17,18]. Serum AMH and inhibin B were assayed using the enzyme-linked immunosorbent assay (ELISA) kit (CUSABIO, Cosmo Bio, Carlsbad, CA). The assay sensitivity for AMH was 0.375 ng/ml, and the intra- and interassay coefficients of variation were 7.1% and 9.8%, respectively. A serum AMH level equal to or lower than 0.17 ng/ml was considered to be in the menopausal range [19]. The assay sensitivity for inhibin B was 7 pg/ml, and the intra- and inter-assay coefficients of variation were 8.9% and 10.5%, respectively.

Continuous data are shown as mean ± SD. Normality in variables was assessed by the Kolmogorov–Smirnov test. Differences between the patients and the control group for continuous variables were assessed with a Student's *t*-test. The difference in the proportion of women having an AMH level in the menopausal range was evaluated using an independent χ^2 test. Correlations between AMH or inhibin B levels and other clinical or laboratory findings were evaluated with a Pearson correlation coefficient. The effect of AITD on AMH or inhibin B levels was assessed using multiple linear regression. Statistical analysis was performed by SPSS software (Statistical Package for the Social Sciences, version 18.0, SSPS Inc., Chicago, IL). A p value of less than 0.05 was considered statistically significant.

Results

The miscellaneous characteristics of the patients and control subjects are demonstrated in Table 1. Steroid, AMH, inhibin B, and gonadotrophin levels and thyroid function tests are shown in Table 2. Patients had at least one type of thyroid antibody positivity: anti-Tg in 51 (60%), anti-TPO in 64 (75.3%), and both in 30 (35%). Two control subjects were excluded from the study becasue of anti-TPO positivity. Patients and controls had regular menstrual cycles. Compared with controls, the AITD group had a significantly lower number of pregnancies and live births (Table 1). The serum levels of gonadotrophins, sex steroids, inhibin B, and free thyroid hormones were similar in the two groups whereas TSH was higher in women with AITD (Table 2). The patients with AITD had significantly lower levels of AMH than controls (Table 2). AMH levels were significantly affected by AITD after adjustment of other potential interfering factors, including age (Table 3 and Figure 1).

The levels of AMH showed a positive correlation inhibin B ($r = 0.268$, $p = 0.001$). Additional significant correlation was not observed between these two hormones and other clinical/laboratory parameters mentioned in Tables 1 and 2.

Table 1. Characteristics of patients with autoimmune thyroid disease and healthy control women, including a detailed data on reproductive history.

	Patients	Control
Age (years)	35 ± 2.9	35.4 ± 2.7
Gynecological age (years)	21.6 ± 3.4	22.4 ± 3.1
BMI (kg/m^2)	27.6 ± 13.9	26.3 ± 5.4
Age at menarche (years)	13.3 ± 1.3	13.1 ± 1.5
Menstrual cycle length (days)	28.7 ± 6.6	30 ± 6.6
Pregnancies (*n*)	2 ± 1.4*	2.5 ± 1.5
Number of live births	1.6 ± 1.1*	2 ± 1

BMI, body mass index. Data are shown as mean ± SD.
*$p < 0.05$ versus the respective control group.

Table 2. Thyroid function tests and serum levels of sex steroids, gonadotrophins, anti-Müllerian hormone (AMH), and inhibin B in patients with autoimmune thyroid disease and control women.

	Patients	Control
FSH (mIU/ml)	7.3 ± 3.6	7.5 ± 3.1
LH (mIU/ml)	6.2 ± 3	5.2 ± 2.1
Estradiol (pg/ml)	48.1 ± 29.6	43 ± 22.3
Testosterone (pg/ml)	0.3 ± 0.2	0.3 ± 0.7
Free testosterone (pg/ml)	1.3 ± 0.6	1.6 ± 0.6
17OH progesterone (ng/l)	1 ± 0.6	1 ± 0.5
DHEAS (μg/dl)	204.6 ± 99.6	186.3 ± 78.8
AMH (ng/ml)	1.16 ± 0.17*	1.28 ± 0.25
İnhibin B (ng/ml)	1.14 ± 0.25	1.10 ± 0.20
TSH (μIU/ml)	2.7 ± 1.1**	2.1 ± 1.2
fT3 (pg/ml)	2.9 ± 0.4	3 ± 0.5
fT4 (ng/dl)	1.3 ± 0.2	1.2 ± 0.2

FSH, follicle stimulating hormone; LH, luteinizing hormone; DHEAS, dehydroepiandrosterone sulfate; AMH, anti-Müllerian hormone; TSH, thyroid stimulating hormone; fT3, free triiodothyronine; fT4, free thyroxine. Normal values: FSH: 2.8–11.3 mIU/ml, LH: 2.4–12.6 mIU/ml, estradiol: 0–160 pg/ml, testosterone: 0.06–0.82 pg/ml, free testosterone: 0.02–3.1 pg/ml, 17OH progesterone: 0.5–3.4 ng/ml, DHEAS: 35–430 μg/dl, fT3: 1.8–4.6 pg/ml, fT4: 0.9–1.7 ng/dl, TSH: 0.27–4.2 μIU/ml. Data are shown as mean ± SD.
*$p = 0.001$ and **$p < 0.001$ versus the respective control group.

Table 3. Multivariate analysis of the effect of autoimmune thyroid disease and other potentially interfering factors on the levels of anti-Müllerian hormone.

	B	SEM (B)	*t*	*p*
Age (years)	−0.001	−0.052	−0.696	0.488
BMI (kg/m^2)	0.008	0.177	2.193	0.030
Duration of AITD	−0.006	−0.139	−1.771	0.079
Pregnancies (*n*)	−0.001	−0.008	−0.100	0.921
TSH	−0.002	−0.014	−0.178	0.859
FSH	0.007	0.110	1.311	0.192
LH	0.002	0.020	0.223	0.824
AITD	0.117	0.263	3.334	0.001

BMI, body mass index; AITD, autoimmune thyroid disease; FSH, follicle stimulating hormone; LH, luteinizing hormone. 'B' refers to the unstandardized coefficients of the estimated regression model and '*t*' refers to the *t* statistics. SEM, standard error of the mean of the coefficients.

Discussion

Our results show that women with AITD have a lower number of pregnancies and live births and lower AMH levels compared with age-matched controls. These findings suggest a diminished ovarian reserve in women with AITD. And to the best of our knowledge, this is the first study to evaluate ovarian reserve in AITD by AMH measurement.

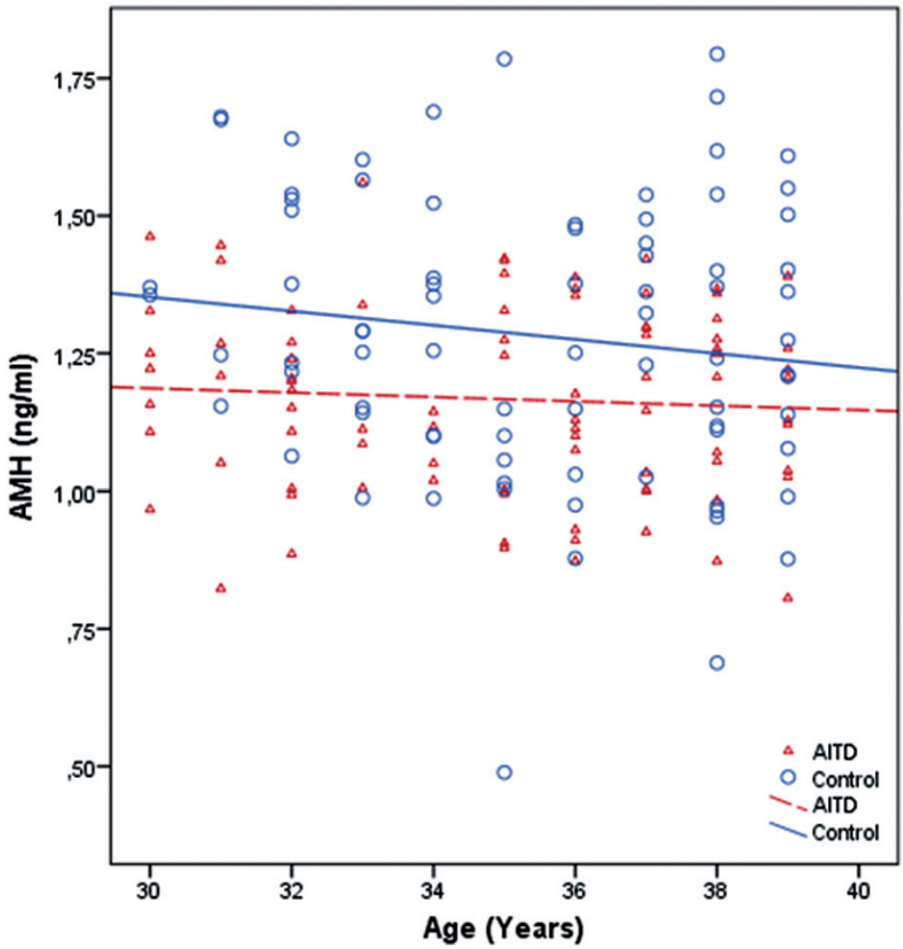

Figure 1. Serum anti-Müllerian hormone (AMH) levels in women with autoimmune thyroid disease and controls.

Although the biological role of AMH in women is still unclear, it is gaining support as a sensitive and non-invasive measure of ovarian function because it signals pool of inactive and initially growing follicles, in other words, the stock of primordial follicles [19]. Compared with FSH, inhibin-B, and E2, AMH has the advantage of reduced variability of its serum concentrations along the menstrual cycle, with consequent uniformity of evaluation [20]. Its serum levels show lower variations between consecutive cycles as well [20]. AMH levels decline after menopause in women with previously regular menstrual cycles [12,13]. In fact the hormone decreases after 30 years of age, a change not observed at the same intensity for FSH, which increases in an insidious manner [21]. AMH was proposed to be the best single marker of the ovarian response to ovulation induction treatment [22].

A relationship between AITD and female infertility has been previously pointed out in several studies. Roussev et al. [6] detected a higher frequency of positive antithyroid antibodies (65% versus 7%) in women reproductive failure than controls. Geva et al. [7] found a significant four-fold increase in the incidence of AITD in women with unexplained and mechanical infertility. In another study, Poppe et al. [8] analyzed 438 couples presenting for the first time to the infertility clinic and 100 fertile control couples matched for age. In couples where infertility was due to female causes, there was an increased risk of associated AITD found in the female partner [relative risk, 2.25; confidence interval (CI), 1.02–5.12; $p < 0.05$]. In this study, the median serum TSH level was significantly higher in patients with infertility, compared with controls (1.30 versus 1.10 mIU/L) [8]. Finally, the prevalence of AITD was three-fold greater in patients with polycystic ovary syndrome than controls [9]. Considering all the studies on this subject, the overall conclusion favors a significantly increased incidence of AITD in women with infertility, with a few exceptions [23].

Multiple mechanisms encompassing both humoral and innate immunity have been suggested to contribute to the higher prevalence of infertility and pregnancy loss seen in hypothyroid women. Thyroid autoimmunity leads to an increase in TSH levels that are well within euthyroid range, but still associated with miscarriage [24]. Potential mechanisms of thyroid antibody-independent effects on TSH are as follows: T cell abnormality, hyperactivity and elevated mass of natural killer cells, polyclonal B cell activation and non-organ-specific autoantibodies, vitamin D deficiency, and concurrent autoimmunity (i.e. endometriosis) [25]. Kelkar et al. [26] showed that human anti-zona pellucida antibodies recognize antigens within murine thyroid tissue. As the zona pellucida and thyroid tissue seem to share similar antigens, the zona pellucida may in turn be a target for anti-thyroid antibodies. This study supported to the ovarian follicle hypothesis and cross reactivity [26].

Our results support previous data that there is an association between AITD and women infertility and our findings suggest the premature ovarian aging as a possible mechanism. A thyroid antibody-dependent effect on TSH may play a role in the pathogenesis as TSH values are higher in our patients than controls although within the euthyroid range. We think that the strength of our study is due to the use of AMH, an early and sensitive marker of ovarian aging [12,13,19–22]. In our study, serum AMH values are positively correlated with inhibin B levels but inhibin B is not significantly different between the two groups. Although inhibin B is thought to provide a direct evaluation of

ovarian reserve, its levels do not show a gradual decline with increasing age. Inhibin B is often viewed as a rather late marker of reduced follicle numbers and a better indicator of ovarian activity than of ovarian reserve, due to its direct link with growing follicles [12,27,28]. A limitation of our study is the absence of antral follicle count (AFC) by USG which would have improved the power of study. But we think that it is not a ''sine qua non'' because AFC is an operator-dependent procedure which is not superior to AMH in the assessment of ovarian reserve [20]. Another limitation is the lack of data regarding the male factor (semen analysis, hormonal evaluation, etc.) and more detailed information on the obstetric history (e.g. the use of *in vitro* fertilization).

In conclusion, data from our study add support to the hypothesis that women with AITD have prematurely aging ovaries. Prospective, randomized, and controlled trials are indicated to confirm these results, to clarify the role of AMH as a marker of ovarian reserve in special populations including women with AITD and to demonstrate the biological significance of our findings with respect to female infertility.

Declaration of interest

The authors report that there are no declarations of interest.

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