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Original Article

Evaluation of the Ovarian Reserve in Adolescents with Hashimoto's Thyroiditis Using Serum anti-Müllerian Hormone Levels

Özalp Akın E et al. Hashimoto's Thyroiditis and Ovarian Reserve

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Abstract
Objective: This study aims to evaluate the ovarian reserve of the adolescent girls with Hashimoto's thyroiditis (HT) with serum anti-müllerian hormone (AMH) levels and compare to healthy adolescents. It is hypothesized that HT decreases ovarian reserve and AMH levels are lower in the HT group.
Method: Thirty HT patients aged between 10-18 years and 30 healthy girls as the control group are enrolled in this cross-sectional study. The mean serum AMH levels of the groups are compared using the Mann-Whitney U test.
Results: There was no statistically significant difference between the patient and the control groups in terms of serum AMH levels. There was negative correlation between serum AMH and thyroid stimulating hormone (TSH) levels and no correlation between serum AMH and anti-thyroid peroxidase (anti-TPO) or anti-thyroglobulin (anti-Tg) levels.
Conclusions: Ovarian reserve of the adolescents by means of serum AMH levels, is not affected by HT according to this study. It is possible that autoimmune damage to the ovaries takes time and adolescence period may be too early on the age spectrum to see the effects, follow up of the patients for reproductive abnormalities and prospective studies are recommended.
Keywords: Hashimoto's thyroiditis, ovarian reserve, Anti-müllerian hormone, adolescents

What is already known on this topic?
Hashimoto's thyroiditis is the most common disease accompanying premature ovarian failure in adult women. In adolescents, there are only two studies examining ovarian reserve of HT patients. AMH levels of adolescent girls with HT were significantly higher than controls in both studies.
What this study adds?
This study there was no statistically significant difference between the HT and the control group adolescents in terms of serum AMH levels. This study enriches the limited existing literature in this topic, exposes two important research questions via secondary findings: association of AMH levels and menarche age, determination of AMH levels according to puberty stage.

Introduction

Hashimoto's thyroiditis (HT) is an autoimmune disease of the thyroid gland characterized by the lymphocytic infiltration of the thyroid gland and is the most common thyroid disorder in children and adolescents. Susceptible individuals who have the combination of abnormalities in the cellular immune responses, auto antibodies, immune susceptibility genes and environmental triggers may develop the disease (1,2).
Anti-müllerian hormone (AMH) is produced by the granulosa cells of the primary follicles, from fetal life to menopause. Serum AMH levels are correlated with a small antral follicle count. AMH is established as a reliable marker for the quantitative evaluation ofovarian reserve due to its level remaining relatively stable during the menstrual cycle and it not being affected by hormonal feedback mechanisms (3-6).

Thyroid hormones take place in control of menstrual cycle, have triiodothyronine sites on oocytes and affect the actions of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) on steroid biosynthesis.
Thyroid dysfunction is associated with menstrual irregularities, anovulation and infertility (7). Premature ovarian failure (POF) describes gonadal failure before the age of 40 with clinical and laboratory findings. Abnormalities of the cellular immunity and autoimmune processes have role in the autoimmune etiology of POF, 80% of the females with idiopathic POF had autoimmune disease in the personal or family history, 50% had high titers of anti-thyroid antibodies, 20% had anti-ovary antibodies (8). HT is the most common disease accompanying POF in adult women (8-10). Even in women with euthyroid HT, the presence of thyroid autoantibodies are related to female infertility (11-13).
In adolescents there are only two studies examining ovarian reserve of HT patients. Results of these two recent studies showed that serum AMH levels of adolescent girls with HT were significantly higher than controls (14, 15). In the current study it is hypothesized that HT decreases ovarian reserve and AMH levels are lower in the HT group.

Material and Method

Thirty adolescents as the patient group and 30 as the control group were included in this study. The patients (aged between 10-18 years) were diagnosed and followed as HT in Doctor Sami Ulus Children's Health and Diseases Training and Research Hospital's Pediatric Endocrinology Outpatient Clinic. Diagnoses of HT were based on clinic evidence, autoantibodies (anti-thyroid peroxidase [anti-TPO] or anti-thyroglobulin [anti-Tg] or both of them should be positive for the diagnosis), hormone levels and ultrasonography findings. At the time of study all the patients had either normal thyroid function or hypothyroidism. The patients with Graves Disease, hyperthyroidism or irregular menstruation cycles were not included in the study. The control group was composed of adolescent girls who were admitted to Doctor Sami Ulus Children's Health and Diseases Training and Research Hospital's Pediatric Outpatient Clinic for minor acute illnesses like upper respiratory tract infections. They did not have any chronic disease, chronic drug use or irregular menstruations in their history. A control appointment was established to assess thyroid function tests, anti-TPO and anti-Tg. Euthyroid and autoantibody-negative 30 age-matched adolescents were included in the control group.
This study protocol is approved by the The Clinical Research Ethics Committee of Zekai Tahir Burak Women's Health, Education and Research Hospital (with the approval number: 75), an informed consent was obtained from all the subjects prior to enrollment.
All the subjects were evaluated for puberty, according to Tanner Staging on physical examination, from stages 1 to 5 (16).
Venous blood samples of the patient and control groups were collected for AMH, thyroid stimulating hormone (TSH), free thyroxin (fT4), anti-TPO, anti-Tg antibody levels. For AMH, blood samples were centrifuged and stored at -20 degrees Celsius and assessed using a AMH Gen II enzyme linked immunosorbent assay (ELISA) kit. According to the manufacturer, the lowest amount of AMH in a sample that can be detected with a 95% probability is 0.08 ng/mL. The patient group was compared with the control group in terms of serum AMH levels.
The study group was evaluated using thyroid ultrasonography for the presence or absence of goiter, thyroid heterogeneity, nodules or any other abnormality by experienced pediatric radiologists. Thyroid volume was measured by the formula using sonographic measurement of three dimensions in centimeters (a,b,c) of each lobe of the thyroid gland:
Thyroid Volume= (a*b*c*0.52)+(a*b*c*0.52)
All the statistical analyses were conducted using the IBM SPSS for Windows Version 21.0 program. Results were presented as mean ± standard deviation or median (minimum-maximum). The categorical variables were shown using numbers and percentages. A Kruskal Wallis test was performed to confirm the difference between >2 groups as non-parametric test. The Mann–Whitney U-test was used as a non-parametric test for a comparison of the numeric variables of the two groups. A Chi-square test was used to confirm the relationship of the categorical variables. Spearman's correlation coefficient was used to determine the relationship between the numeric variables. P values <0.05 were considered statistically significant.

Results

The patient and the control groups had the same mean age which was 14.4 ±1.85 (median age was 15). The mean follow up time of the patient group was 8.5 ±4.5 months. The minimum stage of puberty was 2, median stage was 5 in both groups. There was no significant difference between the groups in terms of pubertal stage and age.
Ten of the 60 subjects (five from the patient group, five from the control group) had not reached menarche at the time of study. Excluding these 10 subjects, the mean menarche age of the patient group was significantly earlier than the control group, (11.4±0.86 versus 12.4±1.04, P=0.001).
Mean serum AMH level of the patient group was 2.18±1.69 ng/ml and the control group was 2.32±1.56 ng/ml, there was no statistically significant difference between the groups (P= 0.784).
There was no statistically significant difference between groups in terms of TSH and fT4 levels. (86 percent of the patient group was on levothyroxine treatment.) Four newly diagnosed cases had subclinical hypothyroidism.

Twenty-six cases were euthyroid. There was no one with overt hypothyroidism. There was a negative correlation between serum AMH and thyroid stimulating hormone (TSH) levels, (in the total sample: r=-0.29, P=0.02, in the study group: r=-0.29, P=0.02, in the control group: r=-0.36, P=0.05). There was no correlation between serum AMH and anti-TPO or anti-Tg levels.

Forty-three of 60 subjects (71%) were at stage 5 puberty and their mean serum AMH level was higher than the mean AMH levels of the other puberty stages 2,3 and 4 which was not statistically significant (2.46±1.74 ng/ml in stage 5 versus 1.76±0.77ng/ml, 1.81±1.52ng/ml, 1.61±1.1ng/ml respectively, P=0.325). (Table 1)

Forty percent of the patient group had goiter, 93.3% had heterogeneity, 43.3% had septation on the thyroid ultrasonography imaging. Goiter, heterogeneity or septation of the thyroid gland were not found to be associated with serum AMH levels.

Discussion

In this study it is found that there is no statistically significant difference in terms of serum AMH levels between the HT adolescents and the control group, serum AMH levels were negatively correlated with serum TSH levels. In a recent study of adults, researchers aimed to evaluate the ovarian reserve of 32 women with HT and compared them to 49 healthy females (17). An unexpected result of this study was that serum AMH levels were higher in woman with HT. The authors explained this finding by polycystic ovary syndrome, which may share a common etiologic linkage with autoimmunity and HT. Another aspect of this study, there was no statistically significant difference between the study and the control patients in terms of antral follicle count.

About ovarian reserve of the HT adolescents two studies exist up to this date. Results of these two recent studies showed that serum AMH levels of adolescent girls with HT were significantly higher than controls. The first one was the research of Pirgon et al (14). They enrolled 30 newly diagnosed HT adolescents as the study group and compared to healthy adolescents which differs from our study. In our study the mean follow up time of the patient group waswas 8.5 ±4.5 months. This means there is a longer time for autoimmune process to affect the ovaries in our study. In Pirgon's study they found no ovarian dysfunction in HT adolescents as menstural irregularity or abnormal FSH, LH levels. The second study in this topic was Erol et al.'s (15). They found higher serum AMH levels and lower serum anti-oxidant levels in euthyroid HT. The discrepancy of serum AMH levels between the current study and Pirgon and Erol's studies may be due to differences in the thyroid status of HT groups and the duration of the autoimmune thyroiditis. But in these 3 studies including the present study it seems that ovarian reserve of the HT patients is not decreased in adolescence in terms of serum AMH levels.

Serum AMH levels are not stable during a woman's lifetime. Hormone expression from the ovaries starts with fetal life, reaching the maximum level at puberty, starts to decrease in adulthood and disappears following the menopause. This hormone is expressed by the granulosa cells of the primary follicles, specifically the preantral and small antral follicles. The expression is decreased in the large antral follicles (18,19). In our study the difference between the serum AMH levels at different puberty stages were not statistically significant but higher AMH levels at puberty stage 5 and lower levels in lower stages, was noteworthy. The number of cases at puberty stage 2,3 and 4 were 5,4 and 8 respectively. But 43 of the 60 cases were at stage 5 puberty. A larger group of subjects are needed to prove a relationship between puberty stage and AMH level which is a major limitation of the present study. Previously, the alternation of serum AMH levels during puberty was evaluated in a well-planned study. Serum AMH levels of 381 girls, aged eight, were recorded. Thirty-nine of them had telarch and their serum AMH levels were significantly lower than the others, (P=0.001). In the longitudinal part of this study, 32 girls were followed and their AMH levels were recorded at seven, nine and 11 years of age. Between seven and nine years of ages, the levels of serum AMH were increased; this was explained by the transition of AMH-silent primordial follicles into AMH-secreting small antral follicles. Between nine and 11 years of age, the levels of serum AMH were decreased, which was explained by the transition of the small antral follicles into less- AMH secreting large antral follicles (20). So we consider that puberty is a special stage of life that may need special AMH levels for puberty stage instead of chronological age.

Serum AMH levels were negatively correlated with serum TSH levels, but not correlated with anti-TPO and anti-Tg levels in the present study. Independent of autoimmunity, subclinical hypothyroidism may affect the ovarian function and reserve. According to the previously mentioned adult HT study, serum AMH and thyroid autoantibodies (anti-TPO and anti-Tg) levels were positively correlated, while serum AMH and TSH levels were not correlated (17). In a large cross sectional study from Belgium women are divided into low, middle and high ovarian reserve categories according to serum AMH levels (21). There was no significant difference in the prevalence of positive anti-TPO antibodies of different ovarian reserve categories compatible with the present study.

Mean menarche age of the patient group was significantly lower than the control group. It is possible that early menarche may be related to early menopause or POF. Knowledge of autoimmunity and early menarche relationship is lacking. In a prospective study, 46 children and adolescents with Hashimoto's thyroiditis were followed up for six years and the mean menarche age was not found to be different from normal children (22). Another autoimmune disorder, Celiac disease, is associated with late menarche age and attributed to autoimmunity and micro and macro nutrient deficiencies (23). In a retrospective study, anti-nuclear antibody

prevalence in postmenopausal women was found to be associated with late menarche age (24). Early menarche is associated with cardiovascular risk factors, obesity, and breast cancer so these patients should be followed for these entities.

This research is one of the first studies conducted about AMH levels of HT patients during childhood. As a pioneer study, the number of subjects is low which is the major limitation of the research. Another limitation was the cross-sectional structure of the study. Long-term follow up is needed to see if there will be any ovarian reserve impairment due to autoimmunity. Currently there are 3 studies investigating the AMH levels of adolescent HT patients, none showed ovarian reserve impairment of adolescents with HT. But follow up time and thyroid status differ in between these studies. More comprehensive prospective studies with higher sample size are required to explain the relationship between HT and ovarian reserve in adolescents

Despite the above limitations the current study enriches the limited existing literature on HT adolescents' ovarian reserve and exposes two other important research areas: association of AMH levels and menarche age, determination of AMH levels according to puberty stage. In conclusion, according to this study the ovarian reserve of the adolescents by means of serum AMH levels, is not affected by HT. It is possible that autoimmune damage to the ovaries takes time and adolescence period may be too early on the age spectrum to see the effects, follow up of the patients for reproductive abnormalities and prospective studies starting from childhood are recommended.

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Table 1. Serum AMH levels according to Tanner puberty stages

Puberty Stage	Number of cases	Serum AMH level (ng/ml)	SD	Median AMH	Minimum AMH	Maximum AMH
Stage 2	5 (8.3%)	1.760	.7797	1.900	.7	2.7
Stage 3	4 (6.6%)	1.815	1.5248	1.100	1.0	4.1
Stage 4	8 (13%)	1.611	1.1852	1.300	.5	4.2
Stage 5	43 (71%)	2.469	1.7464	1.900	.3	8.8