

Received: 2017.01.17
Accepted: 2018.02.07
Published: 2018.02.24

Ovarian Reserve Assessment in Celiac Patients of Reproductive Age

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF G 1 **Erol Cakmak**
B 2 **Savas Karakus**
C 3 **Ozlem Demirpence**
E 4 **Banu Demet Coskun**

1 Department of Gastroenterology, Cumhuriyet University Faculty of Medicine, Sivas, Turkey
2 Department of Obstetrics and Gynecology, Cumhuriyet University Faculty of Medicine, Sivas, Turkey
3 Department of Biochemistry, Cumhuriyet University Faculty of Medicine, Sivas, Turkey
4 Department of Gastroenterology, Kayseri Training and Research Hospital, Kayseri, Turkey

Corresponding Author: Erol Cakmak, e-mail: drecakmak@hotmail.com
Source of support: Departmental sources

Background: This study aimed to investigate ovarian reserve in patients of reproductive age with Celiac disease (CD) using anti-Müllerian hormone (AMH) levels, antral follicle counts (AFCs), and ovarian volume.





Material/Methods: We included into this study 46 CD female patients and 40 healthy female subjects of reproductive age, ages 18–45 years. Venous blood samples were taken from both groups on days 2–4 of the menstrual cycle, and follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2), prolactin (PRL), and AMH levels were measured. On the same day, AFCs and ovarian volumes were determined. Data on body mass index (BMI), gravidity/parity/abortions/alive counts, disease duration, and Marsh histological classification were recorded.

Results: There were no statistically significant differences between CD and control groups in terms of mean age, BMI, or median gravidity/parity/abortions/alive counts ($p>0.05$). Also, there were no statistically significant differences between the 2 groups in terms of mean FSH, LH, E_2 , PRL levels, right and left ovarian volumes, and median right and left ovarian AFCs ($p>0.05$). However, AMH level was significantly lower in the CD group ($p=0.032$). No statistically significant correlation was found between AMH levels and age, BMI, FSH, LH, E_2 , PRL levels, right and left ovarian volumes, right and left ovarian AFCs, or Marsh histological classification using the Spearman correlation test ($p>0.05$). However, an inverse correlation was detected showing that AMH levels decrease with increasing CD duration ($r=-0.054$, $p=0.001$).

Conclusions: We found that AMH level and ovarian reserve was decreased in CD patients of reproductive age compared to healthy controls, and that AMH level and ovarian reserve decreased with increasing disease duration in CD patients.

MeSH Keywords: **Anti-Müllerian Hormone • Celiac Disease • Infertility**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/909033>

 1962  3  —  32



Background

Celiac disease is an autoimmune disease characterized by the involvement of multiple systems, induced by dietary intake of gluten in genetically-predisposed individuals [1]. In celiac disease, gluten-containing nutrients cause decreased enterocyte height, increased intraepithelial T-lymphocytes, crypt hyperplasia, and villous atrophy in small intestine mucosa, and the absorbing surface of the small intestine dramatically decreases and causes insufficient nutrient absorption [2]. As a result, there occur typical intestinal symptoms such as diarrhea, anorexia, abdominal pain, bloating with vomiting, as well as extraintestinal symptoms such as skin disorders, neuromuscular symptoms, bone disease, and reproductive disorders [3].

The prevalence of Celiac disease is especially greater in women of reproductive age. Celiac disease has adverse effects on both fertility and pregnancy in women of reproductive age. This has been associated with autoimmunity and nutrient malabsorption [4]. Celiac disease causes reproductive disorders, including infertility, delayed menarche, amenorrhea, and early menopause in women of reproductive age. In pregnant women, it causes recurrent abortions, preterm deliveries, intrauterine fetal growth restriction, and low-birth-weight infants [4,5]. The cause of these complications in pregnant women is nutritional deficiencies, as well as changed placental functions due to maternal celiac disease auto-antibodies binding to placental transglutaminase and genetic mutations facilitating the formation of microthrombus. Improvement in fertility, miscarriage rates, fetal nutrition, and perinatal course has been observed in these patients upon initiation of a gluten-free diet and recovery of the intestinal mucosa [5,6].

Anti-müllerian hormone (AMH) is a dimeric glycoprotein and member of the transforming growth factor beta family, released by the granulosa cells of pre-antral and early antral follicles. This hormone plays an important role in the regulation of follicle growth [7], and another important function is to slow the reduction of ovarian reserve and to preserve the ovarian reserve. Women of reproductive age with low AMH levels had the lowest ovarian reserve and the highest risk of premature ovarian failure [8]. Therefore, AMH level is the optimal biochemical marker, reflecting the primordial follicle pool and ovarian function. Serum AMH level is recognized as the most useful test for the determination of fertility potential in women of reproductive age. AMH level and primordial follicles decrease with age, and cannot be detected in the menopausal period [9,10].

Our literature search did not find any study exploring the ovarian reserve in celiac patients. The present study aimed to compare ovarian reserves of women with celiac disease of reproductive age and healthy women, using antral follicle counts (AFCs) and ovarian volumes.

Material and Methods

Patients and data collection

Our study included 46 female patients with celiac disease and 40 healthy controls with reproductive potential, ages 18–45 years, between January 2016 and October 2017. Study inclusion criteria were having regular menstrual cycles (length of 21–35 days) and both ovaries being present. Exclusion criteria were smoking, premature menopause, chronic renal disease, chronic hepatic disease, malignancy, history of infertility, hormone therapy affecting ovarian reserve (in the last 6 months), pregnancy, history of surgery, and gynecological problems such as polycystic ovary syndrome.

Subjects included into the study were diagnosed with CD by a combination of medical history, physical examination, serology, and histological analysis of multiple biopsies taken by upper-gastrointestinal endoscopy, and based on ACG guidelines [11]. Duodenal biopsies of the patients with CD were classified based on Marsh-Oberhuber (MO) classification: 0=normal mucosa, 1=increase in intraepithelial lymphocyte count, 2=crypt hyperplasia, 3a=partial villus atrophy, 3b=subtotal villus atrophy, and 3c=total villus atrophy [6].

Venous blood samples were taken from groups on days 2–4 of the menstrual cycle, and follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2), prolactin (PRL), and AMH levels were determined by enzyme-linked immunosorbent assay (ELISA) method. The AMH levels were determined using the AMH ELISA kit (Fine Biological Technology, Wuhan, Hubei, China). FSH, LH, E_2 , and PRL were analyzed by the Chemiluminescent Microparticle Immunoassay (Cobas®, Roche, Mannheim, Germany).

Ovarian volumes and AFCs were determined by pelvic ultrasonography (USG) performed on the same day. Also, subjects' ovarian volumes were calculated by an ellipsoid equation ($\text{length} \times \text{height} \times \text{width} \times 0.526 - 0.526$). Participants' height, weight, and body mass index (BMI), disease duration, gravidity, parity, abortions, and number of alive children were determined. This study protocol was approved by Cumhuriyet University Ethics Committee (No: 2015–11/04) and performed according to the Declaration of Helsinki principles.

Statistical analysis

All statistical analyses were performed using SPSS program 22.0 (Chicago, Illinois, USA). Normality was tested using Kolmogorov-Smirnov test. The *t* test was performed for variables fitting to normal distribution, and the Mann-Whitney *U* test was used for variables not fitting to normal distribution. Spearman correlation test was used for the correlation of AMH

Table 1. Demographic ve clinical characteristics of the patients and controls.

Variables	Control (n=40)	CD (n=46)	p-Value
Age (years)	31.5±9.5	35.2±7.9	0.059*
BMI (kg/m ²)	25.1±3.9	24.6±3.7	0.625*
Gravidity (n)	2 (0–7)	2 (0–7)	0.084**
Parity (n)	1 (0–5)	2 (0–6)	0.159**
Abortions (n)	0 (0–4)	0 (0–5)	0.238**
Alive (n)	1 (0–5)	2 (0–4)	0.140**
Age at CD onset (years)	–	31.5±8.2	–
Age at CD diagnosis (years)	–	32.1±8.2	–
Disease duration (years)	–	4 (1–19)	–
Histology (Marsh classification) (n, %)			
1	–	5 (10.8)	–
2	–	7 (15.2)	–
3	–	34 (74)	–
3a	–	7 (15.2)	–
3b	–	15 (32.7)	–
3c	–	12 (26.1)	–

* Student's t- test; ** Mann-Whitney U-test. BMI – body mass index.

levels with other variables. *p* values <0.05 were regarded as statistically significant.

Results

The mean age of the patients with celiac disease and healthy control subjects were 35.2±7.9 and 31.5±9.5 (*p*=0.059), respectively. There was no statistically significant difference between patient and control groups in terms of mean age, BMI, and median number of gravidity/parity/abortions/alive children (*p*>0.05). The mean disease duration in the CD group was 4 years (range, 1–19 years). Based on CD histological Marsh classification, 5 patients (10.8%) were grade 1, 7 (15.2%) were grade 2, 7 (15.2%) were grade 3a, 15 (32.7%) were grade 3b, and 12 (26.1%) were grade 3c. Demographic and clinical characteristics of the patient and control groups are shown in Table 1.

There was no statistically significant difference between patient and control groups in terms of mean FSH, LH, E₂, PRL levels, right and left ovarian volumes, and median right and left ovarian AFCs (*p*>0.05). However, AMH level was statistically significantly lower in the CD group (*p*=0.032). Laboratory and ultrasound results of the patient and control groups are shown in Table 2.

There was no statistically significant correlation between AMH levels and age, BMI, FSH, LH, E₂, PRL levels, right and left ovarian volumes, and right and left ovarian AFCs using Spearman correlation test (*p*>0.05). There was also no statistically significant correlation between AMH levels and Marsh classification in CD patients (*p*>0.05). However, the decrease in AMH levels with increasing duration of CD disease was found to be highly significant (*r*=–0.504 and *p*<0.001). The result of Spearman correlation test between disease duration and AMH levels is shown in Table 3.

Discussion

This was the first study to assess ovarian reserve in CD and show decrease in ovarian reserve in these patients. Our study assessed ovarian reserve in CD patients and healthy women of reproductive age using AFCs, AMH, and other serum hormone levels (E₂, FSH, LH, and PRL) and ovarian ultrasound. We compared patients with CD and healthy control women in terms of ovarian reserve. As a result of this study, we detected statistically significant decrease in serum AMH level and ovarian reserve in patients with CD compared to healthy women. Moreover, we detected an inverse correlation between disease duration and AMH levels in CD patients. Specifically, we

Table 2. Laboratory result, AMH levels, ovarian volumes, and antral follicle numbers of the groups.

Variables	Control (n=40)	CD (n=46)	p-Value
E ₂ (pg/mL)	80.7±39.5	76.1±23.9	0.972
FSH (IU/L)	8.7±6.7	9.6±8.1	0.681
LH (IU/L)	10.7±5.7	9.4±5.2	0.217
PRL (ng/mL)	13.1±5.4	11.5±5.3	0.061
AMH (pg/mL; median; range)	1619.5 (292–3000)	1184.5 (17–3000)	0.032
Right ovarian volume (cm ³)	4.6 (2.1–10.5)	4.7 (0.8–12.3)	0.945
Left ovarian volume (cm ³)	4.7 (1.9–9.4)	4.9 (1.2–12.4)	0.665
Right ovarian AFC (n)	5 (4–9)	6 (2–15)	0.218
Left ovarian AFC (n)	5 (3–10)	6 (1–13)	0.133

Mann-Whitney U-test. AFC – antral follicle count; AMH – anti-Müllerian hormone; E₂ – estradiol; FSH – follicle stimulating hormone; LH – luteinizing hormone; PRL – prolactin.

Table 3. Correlation analysis of disease duration and AMH levels.

Sperman correlation test	AMH	Disease duration
Serum AMH (pg/mL)	Correlation coefficient	1.000
	Significance	<0.001

AMH – anti-Müllerian hormone.

detected that AMH level and ovarian reserve decreases with increasing disease duration in CD patients.

HLA class II DQ2 or DQ8 genes are predisposing variables in celiac disease and account for about 40% contribute to development of the disease. Furthermore, the development of celiac disease is affected by nutritional factors, including gut microbiota of infants, time and amount of first exposure to gluten, and environmental factors [12]. Being present in and being the alcohol-soluble fraction of gluten taken via nutrients, gliadin has toxic properties. Gliadin triggers inflammatory reaction, especially in epithelial cells of the small intestine lumen, and causes infiltration in lamina propria and atrophy villi [13]. As a result, it causes increase in several celiac-specific auto-antibodies in patients. Combination of celiac-specific serological tests and duodenal biopsy samples is required for diagnosis of celiac disease in adults [14]. As a result of small intestine mucosa inflammation at varying degrees in celiac disease patients, a broad range of classic gastrointestinal symptoms and atypical extraintestinal problems can be present [15]. Being an extraintestinal problem, infertility is highly important, especially in women of reproductive age. The prevalence of celiac disease around the world differs by geographical region; the worldwide prevalence is 1%, and 60–70% of these patients are women of reproductive age [16,17]. Today, the fertility changes caused by celiac disease, together with the possible

adverse course in pregnancy and postpartum period, have begun to be understood better. In women of reproductive age with celiac disease, changes such as delayed menarche, amenorrhea, and premature menopause decrease their fertility. This is caused by autoimmunity, and the nutritional deficiency developing in untreated patients [18]. Studies have shown that infertility and unexplained infertility rates are higher in CD patients than in the general population [15–18]. In another study, 4 (2.7%) CD cases were detected in 150 infertile women and none in healthy women [19]. In a similar study in a northern Sardinia population, CD prevalence was 3.03% in 99 infertile female patients versus 1.06% in the normal population. Also, in this study, the prevalence of celiac disease in women with unexplained infertility, especially in subgroup analysis, was 8% [20]. In a study in infertile Arabic women, CD prevalence was 2.65%, and it was 0.5% in the control group [21]. If female patients of reproductive age have infertility and unexplained infertility, CD investigation is recommended [22]. Kotze detected higher rates of delayed menarche, amenorrhea, and spontaneous abortion in CD patients compared to the control group, regardless of nutritional status [23]. In males and females with celiac disease, recovery of fertility has been observed upon gluten withdrawal and correction of the deficiency of dietary elements with gluten-free diet [24,25]. It is known that immune mechanisms along with nutritional effect play a crucial role in pregnancy-related problems in CD patients [26].

A study in the United Kingdom found that in women with celiac disease, the incidences of infertility, stillbirths, and perinatal deaths were increased and these rates improved after initiation of a gluten-free diet [27].

In a woman of reproductive age, fertility is determined by ovarian reserve, which represents both the quality and the quantity of the ovarian follicular pool. It has been reported that ovarian reserve decreases with age and in some autoimmune diseases. The best test used in clinical practice to assess ovarian reserve is the AMH test [28]. In the literature, no study was found on the effect on AMH levels in celiac disease patients. Recently, however, several studies have explored the relationship between some of the autoimmune diseases, and fertility and ovarian reserve in women of reproductive age. In their study in women of reproductive age with Behcet's disease, Mont'Alverne et al. detected decreased AMH level and ovarian reserve, and they reported that Behcet's disease activity contributes to the decrease in ovarian reserve [29]. In a study in female patients with systemic lupus erythematosus, it was reported that AMH levels are decreased in this disease compared to the healthy population, and active disease has negative effect on ovarian reserve [30]. In another study, a significant decrease was observed in AMH levels in female patients of reproductive age with Crohn's disease compared to the control

group [31]. In FMF patients with M694V mutation, a decrease was detected in AMH levels [32]. These studies show that autoimmune diseases have adverse effects on fertility. Especially, there is an inverse correlation between autoimmune disease activity and ovarian reserve. AMH levels and ovarian reserve decrease with increasing autoimmune disease activity.

The limitation of our study is the low number of patients; however, this is the first study in the literature assessing the ovarian reserve in CD patients of reproductive age. Our findings need to be supported by larger studies.

Conclusions

We detected decreased AMH levels and ovarian reserve in CD patients of reproductive age compared to the healthy control group. Moreover, we detected that AMH levels and ovarian reserve decrease with increasing disease duration, and there is an inverse correlation between them.

Conflicts of interests

None.

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