



## Assessment of ovarian reserve in patients with migraine

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### Abstract

#### Objective

The aim of our study is to investigate the relationship between migraine and ovarian reserve.

#### Methods

The study group consists of women between the ages of 25-51, including 44 patients diagnosed with migraine and 43 controls. Ovarian reserves were performed by antral follicle count and measured anti-Müllerian hormone level.

The cohort was divided into four subgroups according to age as follows: 30 years and below, 31-35 years, 36-40 years, and 41 years of age and above.

#### Results

Of the 87 individuals included in this study, 44 were migraine patients, and 43 were healthy controls. The mean ages of the study and control groups were 34.3 (minimum: 25, maximum: 51) and 36.5 (minimum: 27, maximum: 51) years, respectively. There was no statistically significant difference between the two groups regarding age ( $p=0.48$ ). In the study and control groups, respectively; mean AMH levels were  $2.67 \pm 2.46$  ng/mL and  $2.55 \pm 2.38$  ng/mL ( $p=0.819$ ), mean basal FSH levels were  $7.92 \pm 2.52$  U/L and  $9.11 \pm 3.19$  U/L ( $p=0.066$ ), mean basal LH levels were  $6.35 \pm 3.59$  U/L and  $6.06 \pm 2.86$  U/L ( $p=0.681$ ), mean basal estradiol levels were  $65.02 \pm 69.54$  ng/L and  $49.47 \pm 27.08$  ng/L ( $p=0.244$ ), and mean AFC were  $10.9 \pm 3.9$  and  $10.2 \pm 3.7$  ( $p=0.435$ ). Between subgroups aged  $\leq 30$  years, serum anti-Müllerian hormone levels were found to be significantly different ( $p=0.036$ ). There was no statistically significant difference between any age subgroups in terms of antral follicle count.

#### Conclusions

In conclusion, detecting possible reduction of ovarian reserves in reproductive-age (especially younger than 30 years) migraine patients by utilizing anti-Müllerian hormone and ultrasonographic markers would allow these women to make cognizant decisions regarding marriage and family planning, as well as inform them whether they are in early menopause risk

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## Introduction

Migraine is a headache attack lasting for 4-72 hours that hinders or disrupts routine activities, and may be accompanied by various neurological, gastrointestinal and autonomic symptoms such as photophobia, phonophobia, nausea and vomiting.<sup>1,2</sup>

Migraine causes considerable deterioration in quality of life.<sup>2</sup> In 2016, the World Health Organization listed migraine second among disorders that contribute to lost productivity.<sup>1,2</sup> Diagnosis is based on the characteristics of headache and associated symptoms.<sup>2</sup> Two clinical syndromes of the disorder are defined as migraine with aura and without aura.<sup>3</sup> A migraine attack may comprise prodrome, aura, headache and postdrome phases.<sup>2</sup>

Prevalence of migraine varies according to age, gender, ethnicity and income.<sup>2</sup> While frequency of attacks is similar between sexes in children and elderly, it doubles in women compared to men following puberty, and frequency of attacks is higher in women throughout reproductive years than that in men.<sup>1</sup>

Sex hormones may function as major modulators considering the distinct effects of migraine in men and women.<sup>1</sup> Estrogen plays a key role in migraine; fluctuations in estrogen levels during puberty, menstruation, pregnancy, menopause and postmenopause influence migraine.<sup>1</sup> Menstruation, oral contraceptives, hormone replacement therapy, pregnancy and menopause are known to affect the course of migraine.<sup>2,3</sup>

Migraine prevalence is highest during the late menopausal transition, especially in women that experience premenstrual stress disorder.<sup>4</sup> Among mechanisms in which perimenopause can trigger migraine are "estrogen deficiency", fluctuations in ovarian hormones and, indirectly, increase in the number of accompanying disorders.<sup>4</sup>

The modern age has brought dramatic changes in social and behavioral life.<sup>5</sup> There is a tendency in women to postpone pregnancy and childbirth until their thirties or even forties.<sup>5</sup> However, this tendency leads to various issues, especially decrease in conception probability.<sup>5</sup> As assisted reproductive technologies are put to use at that point, it is important to accurately determine the ovarian reserve.<sup>6</sup> The term "ovarian reserve" has been traditionally used to describe the reproductive potential of women, especially the number and quality of their oocytes.<sup>6,7</sup> Ovarian aging is reduction in the ovarian follicle pool, and

decline in quality and quantity of oocytes with advanced age.<sup>8,9</sup>

The reduction in a woman's ovarian reserve over time is irreversible, and decrease in the number of primordial follicles is indicative of infertility or menopausal transition.<sup>6</sup> While anti-Müllerian hormone (AMH) levels and antral follicle count (AFC) are currently the preferred methodology for evaluating ovarian reserves, levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol between the second and fourth days of the menstrual cycle may also be utilized in conjunction.<sup>6</sup>

Anti-Müllerian hormone, which is a homodimeric glycoprotein belonging to the transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily, was initially described by Alfred Jost in the 1940s in terms of its role in men's sexual differentiation.<sup>5,10</sup> The gene for AMH is located on chromosome 19 (19p13.2-13.3), its molecular weight is 140 kDa and two serine/threonine kinase transmembrane receptors mediate its signaling pathway.<sup>10,11</sup>

As AMH is expressed during normal early folliculogenesis, its levels are relatively independent of circulating gonadotropins at physiologic levels, which allows for testing at any stage of the menstrual cycle.<sup>6</sup> Anti-Müllerian hormone is secreted by the granulosa cells of primary, secondary, pre-antral and small antral follicles (<6 mm) into the follicular fluid and the bloodstream.<sup>5,9</sup> When follicles reach a later developmental stage and become large enough to be selected as dominant follicles, transcription of the AMH gene ceases.<sup>5</sup>

Secretion of AMH peaks at the neonatal stage, before the onset of puberty and around the age of 24.5 years. AMH levels then gradually diminish until menopause.<sup>10</sup> Serum AMH levels decrease approximately 5.6% per year and become undetectable three to five years before menopause onset.<sup>10</sup> Evidence that AMH testing is the most practical and reliable method for evaluating ovarian reserves in various clinical settings has been mounting steadily.<sup>11,12</sup>

The aim of our study is to investigate the relationship between migraine and ovarian reserves.

## Methods

Among patients that visited the Neurology Department of Antalya Education and Research Hospital (AERD),



those between 25 and 51 years of age, and diagnosed with chronic migraine according to the International Classification of Headache Disorders 2<sup>nd</sup> Edition (ICHD-2) beta version with episodic aura or without aura were included in the study. Patients' demographic characteristics and results of examination for headache were recorded in accordance with the AERD Neurology Department Record Form for Headache Patients. Time since migraine onset, monthly frequency, attack duration, whether migraine is associated with menstruation, painkiller usage, prophylactic medication usage, and accompanying signs and symptoms (nausea, vomiting, sensitivity to sound, light, smell or physical activity, loss of appetite, etc.) were the parameters inquired from the patients.

In order to measure the disabling impact of headaches on patients' lives, the Migraine Disability Assessment (MIDAS) Test was applied and the number of days with migraine within the last month was recorded. The Visual Analogue Scale (VAS) was used for quantifying the intensity with which the patients experienced headache.

For assessment of ovarian reserves, the patients were transferred to the Obstetrics and Gynecology Department, where their ovarian volume and number of antral follicles were measured using ultrasonography. On the same day, 5.0 mL of venous blood sample was drawn from the patients into separator gel collection tubes with coagulation activator (Becton Dickinson Vacutainer SST II Advance Plus, lot 7163845, Plymouth, UK). Following centrifugation at 3,000 rpm for 10 minutes, serum samples were transferred into secondary tubes and stored at -80°C until analysis. AMH levels were measured with a commercially available paramagnetic particle-based chemiluminescence immunoassay kit (Beckman Coulter, Inc., USA) using a DXI 800 Analyzer (Beckman Coulter, Inc., USA).

The control group comprised healthy women that visited the hospital for family planning counseling. The study group included 44 patients, while 43 individuals constituted the control group.

**Statistical Analysis**

IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) software package was used for statistical analysis of resulting data. Mean, standard deviation and minimum/maximum values were given when reporting descriptive statistics. Variables were tested to determine whether they were normally distributed.

The Student's t-test was applied to AMH levels and follicle counts, which exhibited normal distribution. Since nonparametric distribution was observed when the study and control groups were arranged according to age and serum AMH levels, the Mann-Whitney U test was utilized. A p value of <0.05 with 95% confidence intervals was regarded as statistically significant.

**Results**

Of the 87 individuals included in this study, 44 were migraine patients and 43 were healthy controls. Demographic and laboratory parameters are given in Table 1. Mean ages of the study and control groups were 34.3 (minimum: 25, maximum: 51) and 36.5 (minimum: 27, maximum: 51) years, respectively. There was no statistically significant difference between the two groups in terms of age (p=0.48). In the study and control groups, respectively; mean AMH levels were 2.67 ± 2.46 ng/mL and 2.55 ± 2.38 ng/mL (p=0.819), mean basal FSH levels were 7.92 ± 2.52 U/L and 9.11 ± 3.19 U/L (p=0.066), mean basal LH levels were 6.35 ± 3.59 U/L and 6.06 ± 2.86U/L (p=0.681), mean basal estradiol levels were 65.02 ± 69.54 ng/L and 49.47 ± 27.08 ng/L (p=0.244), and mean AFC were 10.9 ± 3.9 and 10.2 ± 3.7 (p=0.435) (Table 1).

**Table 1.** Demographic and laboratory parameters in the study cohort

		n	Mean	Std. Deviation	min	max	p
<b>Age</b>	Migraine	44	34.30	4.58	27.00	51.00	0.480
	Control	43	36.49	5.53	25.00	51.00	
<b>AMH (ng/mL)</b>	migraine	44	2.67	2.46	0.13	14.67	0.819
	Control	43	2.55	2.38	0.08	14.85	
<b>FSH</b>	migraine	40	7.92	2.52	3.31	14.97	0.066
	Control	43	9.11	3.19	4.66	21.58	
<b>LH</b>	migraine	40	6.35	3.59	2.44	17.12	0.681
	Control	43	6.06	2.86	1.48	13.32	
<b>E2</b>	migraine	40	65.02	69.54	14.00	361.00	0.244
	Control	43	49.47	27.08	17.00	126.00	
<b>AFC</b>	migraine	43	10.93	3.95	4.00	22.00	0.435
	Control	43	10.28	3.74	1.00	16.00	

The study and control cohorts were divided into four subgroups according to age as follows: 30 years and below, 31-35 years, 36-40 years, and 41 years and above. Between subgroups aged ≤30 years, serum AMH levels were found to be significantly different (p=0.036). There was no statistically significant difference between any age subgroups in terms of AFC (Table 2).



**Table 2.** Comparison of the anti-Müllerian hormone levels and antral follicle counts by age groups

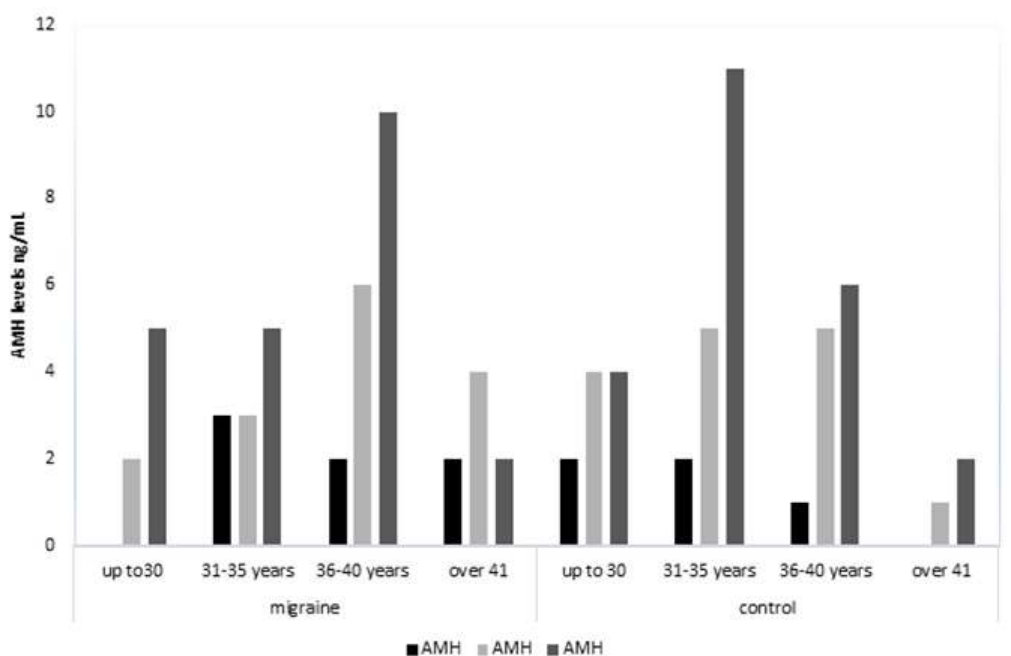
		Age (years)	n	Mean	Mean Rank	p
AMH	migraine	up to 30	7	4.19	12.70	0.036
	control	up to 30	10	2.93	6.85	
AFC	migraine	up to 30	6	14.70	10.92	0.112
	control	up to 30	10	10.80	7.05	
AMH	migraine	31-35	11	2.30	13.86	0.574
	control	31-35	18	2.70	15.69	
AFC	migraine	31-35	11	12.18	17.00	0.318
	control	31-35	18	10.30	13.78	
AMH	migraine	36-40	18	2.84	15.78	0.832
	control	36-40	12	2.15	15.08	
AFC	migraine	36-40	18	9.60	15.97	0.717
	control	36-40	12	9.30	14.79	
AMH	migraine	over 41	8	1.48	5.50	0.414
	control	over 41	3	2.04	7.33	
AFC	migraine	over 41	8	9.10	5.31	0.257
	control	over 41	3	12.00	7.83	

Migraine patients and healthy controls were subgrouped according to AMH levels for comparison. AMH levels below 0.70 ng/mL were categorized as deficient, 0.71-2.00 ng/mL as low, and above 2.01 ng/mL as sufficient. In deficient, low and sufficient subgroups, p values were found to be 0.808, 0.917 and 0.314, respectively, indicating no significant difference. Distribution of the study subjects according to age and AMH levels is shown in Figure 1.

## Discussion

Ovarian reserves of reproductive age women diagnosed with migraine were assessed in this study. Migraine is a disorder commonly suffered by women. There are papers reporting that migraine is observed twice as frequently in women being treated at infertility clinics.<sup>13</sup> Moreover, studies have shown that migraine attack frequency is higher during the perimenopausal stage and menopause.<sup>14,15</sup> While the average age of menopause onset is 51 in the USA, perimenopause starts ten years before menopause (from the middle to the end of thirties in some) and lasts until the end of reproductive years.<sup>15,16</sup> Fluctuations in ovarian functions and ovarian hormone levels result in certain symptoms in this transition period.<sup>15</sup> Such chaotic hormonal variation may cause more frequent and much severe headaches, as well as return of migraine attacks in patients with quiescent disease course.<sup>15</sup>

The “estrogen deficiency” hypothesis, which was proposed approximately 40 years ago and is still widely accepted today, offers an explanation as to how migraine is triggered by ovarian hormones.<sup>17,18</sup> In accordance with this hypothesis, migraine attacks are induced due to the rapid decrease in estrogen levels just before menstruation and during menopausal transition or in the early postmenopausal period.<sup>17</sup>



**Figure 1.** Comparison of the age groups of migraine and healthy controls according to deficient (below 0.70 ng/mL), low (0.71-2.00 ng/mL) and sufficient (above 2.01 ng/mL) AMH levels.



Symptoms of menopause include hot flashes, night sweats, sleeping problems, as well as headaches. Low levels of AMH are linked with higher prevalence of symptoms during the menopausal period.<sup>19</sup> Low AMH levels, high FSH levels and low AFC are associated with menopause onset.<sup>16,20</sup>

Several studies have established the strong relationship between AMH and timing of natural menopause.<sup>20,21</sup> Low levels of serum AMH are considered to be an indication of reduced ovarian reserve.<sup>22</sup> In a study conducted by Fong et al.<sup>22</sup>, serum AMH levels were found to constitute an accurate diagnostic marker for predicting ovulatory dysfunction in women with premature ovarian insufficiency.<sup>22</sup> A prospective study by Steiner et al.<sup>23</sup> showed that women aged 30-44 years with AMH levels below 0.7 ng/mL had lower fecundability compared to women with higher AMH levels.<sup>23</sup>

Tehrani et al.<sup>21</sup> used a statistical model based on serum AMH levels to predict the age at menopause for different age groups of reproductive-age women.<sup>21</sup> AMH levels are relatively stable from one menstrual cycle to another and have a high intraclass correlation coefficient, which means that a single measurement can serve as reliable estimation for a given individual.<sup>21</sup>

Nair et al.<sup>20</sup> reported that AMH levels could be utilized to estimate the risk of menopause within five years in women in their late reproductive years. Particularly, they found that AMH levels higher than 2.0 ng/dL were especially associated with very low menopause risk and that undetectable levels of AMH in women in their forties indicated much higher risk of menopause.<sup>20</sup>

In our study, mean serum AMH levels of the migraine patients (mean age  $34.29 \pm 4.58$  years) and the control group (mean age  $36.49 \pm 5.53$  years) were  $2.67 \pm 2.46$  ng/mL and  $2.55 \pm 2.38$  ng/mL, respectively (Table 1). Mean AMH levels of both groups were higher than 2 ng/mL, suggesting that the menopause risk within five years is low. Nonetheless, closer inspection of the data revealed that 22 patients (50%) in the migraine group had AMH levels below 2 ng/mL, while 15 (34%) had levels below 1.5 ng/mL. Among the control group, AMH levels of 20 individuals (46%) were below 2 ng/mL and those in 15 (35%) were below 1.5 ng/mL. The number of subjects with AMH levels of <0.7 ng/mL, which is criterion used in the study conducted by Steiner et al.<sup>23</sup>, was 7 (15.9%) in the migraine group and 5 (11.6%) in the control group. In light of these data and the fact that ages of the participants in our study ranged broadly (minimum age was 27 years

and maximum age was 51 years in the migraine group, whereas those in the control group were 25 and 51, respectively), we divided our subjects into subgroups of five years of age. While comparison of other age subgroups yielded no statistically significant difference, AMH levels of the migraine and control subgroups aged below 30 years were significantly different (Table 2).

Waziri and Omoti<sup>13</sup> stated that the cause of migraine headaches suffered by many of the women treated at their center was infertility and that migraine was twice as common in infertile women. The age of migraine onset was reportedly five years earlier in women with infertility issues.<sup>13</sup>

Spontaneous premature ovarian failure or primary ovarian insufficiency (POI) is seen in one in 1,000 women below the age of 30 and one in 100 women below the age of 40.<sup>24</sup> Low serum AMH levels have been identified as an early marker of reduced ovarian reserves.<sup>22</sup> In women with POI, serum AMH is an accurate diagnostic marker for predicting ovulatory dysfunction.<sup>22</sup>

AFC is the total number of follicles in both ovaries as counted using ultrasound in the early follicular phase of the menstrual cycle (day 2-4).<sup>6</sup> The number of follicles smaller than 10 mm is regarded as a representation of ovarian reserve.<sup>25</sup> While AFC is easy to perform and produces rapid results, differences in technical specifications of ultrasound devices, as well as differences in experience and skill levels of the professionals carrying out the count lead to disparities in counts made at different facilities and different times.<sup>6</sup> In our study, no statistically significant difference was observed between AFCs of the patient and control groups.

There are numerous papers in the literature about the relationship between AMH levels and ovarian reserves in various clinical settings.<sup>26-29</sup> To our knowledge, ovarian reserves in migraine patients have not been investigated previously, making our study the first one addressing this matter. Furthermore, the immunoassay technique used for measuring AMH levels in our study produces more sensitive results compared to the ELISA method. The major limitation of our study was the limited number of study subjects. Therefore, a larger cohort that particularly consists of young migraine patients in their reproductive years would be recommended for evaluation of ovarian reserves. Standardization for interpreting AMH levels is yet to be established due to the lack of commonly accepted reference ranges, absence of a definite cut-off value corresponding to insufficient ovarian reserves and



different measuring techniques used in different studies. An international harmonization effort is needed for AMH.

In conclusion, detecting possible reduction of ovarian reserves in reproductive-age migraine patients by utilizing AMH and ultrasonographic markers would allow these women to make cognizant decisions regarding marriage and family planning, as well as inform them whether they are in early menopause risk. Future studies on this subject involving larger cohorts would be instructive.

**Authors contribution:** GA, designed the study, reviewed and criticized the manuscript; NU and AU, performed data collection; GA and AY, designed the study, performed statistical analysis, drafted, criticized, and reviewed the manuscript. All authors read and approved the final manuscript.

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