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Anitmullerian Hormone and the Perimenopausal Symptoms

Pallavi Sharma*, Indrani Ganguli, Sangeeta Chaudhury, Mamta Dagar and Mala Srivastava

Clinical Associate, Institute of obstetrics and Gynaecology, Gripmer, New Delhi

***Corresponding Author:** Pallavi Sharma, Clinical Associate, Institute of obstetrics and Gynaecology, Gripmer, New Delhi.

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Introduction

Menopauseis the stage when permanent cessation of menstruation occurs following loss of ovarian activity. The term menopause is derived from the Greek words, *men* (month) and *pausis* (cessation). The estimated mean age of menopause is 46 years in India, and is lower than that of the Caucasians.1The years prior to menopause that encompass the change from normal ovulatory cycles to cessation of menses are known as the Perimenopausal transitional years, marked by irregularity of menstrual cycles.

Climacteric indicates the period of time when a woman passes from the reproductive stage of life through the Perimenopausal transition and the menopause to the postmenopausal years. This change tends to occur over a period of years, and is a consequence of biological aging. The average age of onset of menopause after the Perimenopausal transition is 4-5 years. The transition is considered to be over, once a woman has attained 12 months of amenorrhea.

There are no specific tests that can predict menopause. Till date there are numerous ways to detect the ovarian reserve, both hormonal and biochemical [2]. Most of these tests have limitations either in their sensitivity or their accuracy. In fact, combined markers are used that include subject's chronological age, basal FSH, inhibin B, antral follicle count, ovarian volume etc. Many studies have concluded that, as a single biomarker, AMH is the best biomarker for ovarian reserve [3-5] and its uniqueness lies in the fact that it does not vary as per the day of the cycle.

Since AMH is a direct reflector of the ovarian follicular cohort so it is likely that exhaustion of the follicular pool will lead to cessation of biological activity of the ovary and decrease in the AMH levels. This will make a woman experience signs and symptoms of menopausal transition. This study made an attempt to find the normative values of AMH in the study group and correlate the levels with the menopausal symptoms.

Aims and objectives

To determine Antimullerian hormone levels in symptomatic Perimenopausal women and to correlate the values further with the Perimenopausal symptoms.

Inclusion Criteria

1. Menstrual problems. (Women with irregular cycles)
2. Vasomotor symptoms. (Hot flashes and night sweats)

3. Genitourinary problems. (Urinary frequency, vaginal dryness)
4. Musculoskeletal symptoms. (Muscular and joint pains)
5. Other symptoms (Insomnia, mood changes, depression, dry skin and decreased libido)

Exclusion Criteria

1. Natural or surgical menopause.
2. Women with past history of infertility.
3. Previous genital and extra genital malignancies.
4. Women who have undergone chemotherapy or radiotherapy.

Material and Methods

The study was done from Jan 2011-Dec 2012 in Sir Ganga Ram hospital New Delhi. A total of hundred women from 35-55 years visiting the outpatient department were studied. Exclusion and inclusion criteria were seen before enrolling the woman in this study. Women were counseled regarding the usefulness of this study, questionnaire based performas were filled up by the patients and samples were taken on any day of the menstrual cycle after getting informed consents.

The results were disclosed to the patients after the completion of this study and women were counseled based on AMH results. Sample size was calculated using the formula for descriptive study ($Z^2 \times p \times q)/d^2$). When the estimated prevalence of Perimenopausal symptoms in women (p) = 50%, precision error of estimation (d) = 0.10 (or 20% of p), and alpha = 0.05, a sample size of 100 cases is needed.

Data collection

The purpose of the study was explained to the patient in the language best understood by her. Written and informed consent were taken. The test of Antimullerian hormone was tested with 2ml fasting sample in EDTA vacutainer not related to the menstrual cycle. It was tested using ELISA technique. The blood sample was centrifuged at 4500 rpm for 5 min using a centrifuge (Haraeus Biofuge Stratos, Thermo scientific) to obtain the serum and stored at -20°C until further used.

AMH ELISA involves thawing of chemicals, controls at 4°C and samples at RT, 1 hour before use. The reagents were thawed to RT and mixed thoroughly by gentle inversion before use. The ELISA was performed as per the manufacturers protocol (AMH Gen II ELISA Kit – A79766/A79765) Micro titration strips were marked priorly. In brief, 20 µl of calibrators (controls)/samples were added to a 96-well plate (coated with AMH antibody). 100 µl of the AMH Gen II assay buffer [consisting of buffer with BSA, protein (bovine, mouse), <0.3% ProClin 300 and sodium azide] was added to each well and incubated at RT , shaking at 300-600 rpm for 1 hour in orbital micro-plate shaker as per manufacturer's protocol, wash the plate 5 times with 1X wash buffer (from wash Concentrate I).

Further, 100 µl of the antibody-biotin conjugate solution was added to each well and incubated for 1 hour. The plate was washed again as mentioned above. Added 100 µl of Streptavidin-enzyme conjugate was added to each well and incubated for 30 minutes at room temperature with shaking and after washing, 100 µl of the TMB substrate, a chromogen was added and incubated for 30 minutes. The wells were then read at 450 nm with 650 nm as reference using ELISA Plate Reader-Infinite® M200 from Tecan Instrument

Data analysis Statistical testing was conducted with the statistical package for the social science system version SPSS 17.0. Continuous variables are presented as mean ± SD, Median (min-max), and categorical variables are presented as absolute numbers and percentage. Since the distribution of AMH was not normal, Mann Whitney U test was used to compare continuous variables between two groups and Kruskal Wallis test was used for more than two groups. $P < 0.05$ was considered statistically significant.

Results

Maximum ladies in the study group were between 35-40 years and only seven women were above 50 years of age (Table 1). The values of AMH were found to be inversely proportionate to age, which was a statistically significant association. P value 0.03 (Table 2).

The symptoms were grouped under five headings, Musculoskeletal (muscular and joint pains) (63%), vasomotor (hot flashes and night sweats) in 66%. Genitourinary symptoms were seen in 80% women and 93% had other symptoms (insomnia, mood changes, depression, and dry skin and decreased libido) menstrual irregularities were witnessed by 42% women (Table 3)

Age Groups	Frequency	Percentage
35-40	44	44%
41-45	34	34%
46-50	15	15%
50-55	7	7%
Total	100	100%

Table 1: *Age distribution of women.*

Age Groups	N = 100	Mean	Std. Deviation	Minimum	Maximum	P value
35-40	44	0.465	0.803	0.000	2.875	0.034
41-45	34	0.177	0.455	0.000	2.131	
46-50	15	0.032	0.088	0.000	0.326	
50-55	7	0.023	0.060	0.000	0.158	
Total	100	0.271	0.619	0.000	2.875	

Table 2: *Correlation of AMH between different age groups.*

Complaint	Frequency	%
Musculoskeletal	63	63%
Vasomotor	66	66%
Genitourinary	80	80%
Others	93	93%
Menstrual Irregularities	42	42%

Table 3: *Correlation of Perimenopausal symptoms with age groups.*

Almost all women had more than one compliant and the ones studies under grouped as others which included Insomnia, mood changes, depression, dry skin and decreased libido were seen the most. Menstrual irregularities were lowest in all age groups (Table 4). The results showed that 61% women complained of dyspareunia and vaginal dryness, Hot flashes were seen in 59% women, muscular pains were seen in 55%, decreased libido was seen in 54%, mood changes were seen in 53% women, 48% women had menstrual irregularities, 46% complained of joint pains and 40% had night sweats. Dry skin was seen in 35% women and 33% complained of urinary frequency .Insomnia was witnessed in 30% and 25% complained of depression (Table 5).

Further comparisons were made using these symptoms with the mean AMH in the symptomatic and the asymptomatic groups. Night sweats were seen in 40 women who had mean AMH levels 0.011 ng/ml whereas 60 women who did not have night sweats had a mean AMH0 0.445 ng/ml. The comparison was statistically significant. (P value 0.001) 61 women with vaginal dryness had a mean AMH of 0.096ng/ml whereas asymptomatic ones (n = 39) had mean AMH of 0.545 ng/ml. This comparison was statistically significant (P value 0.001).

Age Groups	Total N = 100	Others	Genitourinary	Vasomotor	Musculoskeletal	Menstrual Irregularities
35-40	44	39	31	26	23	13
41-45	34	32	27	22	20	12
46-50	15	15	15	11	14	11
50-55	7	7	7	7	6	6

Table 4: *Distribution of various symptoms observed in the study group.*

Complaint		Frequency	%
Genitourinary	Dyspareunia	61	61%
	Urinary Frequency	33	33%
	Vaginal Dryness	61	61%
Vasomotor	Night Sweats	40	40%
	Hot Flushes	59	59%
Musculoskeletal	Joint Pain	46	46%
	Muscular Pain	55	55%
Others	Decreased Libido	54	54%
	Mood Changes	53	53%
	Dry Skin	35	35%
	Insomnia	30	30%
	Depression	25	25%
Menstrual Irregularities		42	42%

Table 5: *Frequency of Various Symptoms.*

Urinary frequency was reported in 33 women with the mean AMH was 0.136, whereas it was 0.338ng/ml in 67 asymptomatic women. This observation was statistically insignificant (P value 0.456). A statistically significant association (p = 0.001ng/ml) was seen in women with dyspareunia (n = 61) with AMH of 0.129 ng/ml when compared to 39 asymptomatic women mean AMH of 0.493 ng/ml. Muscular pain was seen in 55 women with mean AMHof0.177ng/ml and 45 women who did not have this complaint had an AMH of 0.387 ng/ml.

This observation was statistically significant (p value 0.002).Joint pain were seen in 46 women with mean AMH0.149ng/ml whereas women free of the complaint (n = 54) had an AMH 0.375 ng/ml. This observation was statistically significant (p value 0.051). A statistically insignificant association was seen between AMH and insomnia (p = 0.148ng/ml), women with insomnia (n = 30) had AMH of 0.162ng/ml whereas asymptomatic ones (n = 70) had a mean AMH of 0.318ng/ml.

Mood changes were correlated with AMH, 53 women with mood changes had a mean AMH of 0.147ng/ml and asymptomatic (n = 47) had AMH0.411 ng/ml. This observation was statistically insignificant (p value 0.145). Depression was reported in 25 women with mean AMHof 0.436ng/ml whereas 75 women who did not have this problem had mean levels of 0.216ng/ml. The observation was statistically insignificant.35 women reported to have dry skin with the mean 0.136ng/ml and in 65 women said no for this with mean AMH 0.344 ng/ml. This observation was statistically insignificant (p = 0.368).Decreased libido was observed in 54 women with mean AMH as 0.289ng/ml whereas 46 asymptomatic ones had AMH 0.250 ng/ml. This result was statistically insignificant (P = 0.141).

When AMH was correlated with menstrual irregularity it was seen that women with irregular cycles (n = 42) had mean AMH of0.173 ng/ml and those with regular cycles (n = 58) had mean level 0.342 ng/ml. (P = 0.037).This was a statistically significant association (Table 6).

Complaint	Symptomatic (n = 100)	Mean AMH Ng/ml	SD	Median	P value
Hot flushes	Yes59	0.0576	0.252	0.000	<.001*
	No 41	0.059	0.833	0.175	
Night sweats	Yes 40	0.445	0.040	0.000	<.001*
	No 60	.011	0.752	0.134	
Vaginal dryness	Yes 61	0.096	0.362	0.000	<.001*
	No 39	0.545	0.815	0.169	
Urinary frequency	Yes 33	0.136	0.335	0.000	0.456
	No 67	0.338	0.712	0.000	
Dyspareunia	Yes 61	0.129	0.438	0.000	<.001*
	No 39	0.493	0.782	0.142	
Muscular pain	Yes 55	0.177	.517	0.000	<.002*
	No 45	0.378	0.714	0.064	
Joint pains	Yes 46	0.149	0.390	0.000	0.051
	No 54	0.375	0.750	0.000	
Insomnia	Yes 30	0.162	0.481	0.000	0.148
	No 70	0.318	0.667	0.000	
Mood changes	Yes 53	0.147	0.411	0.000	0.0145
	No 47	0.411	0.772	0.000	
Dry skin	Yes 35	0.136	0.318	0.000	0.368
	No 65	0.3440	0.723	0.000	
Depression	Yes 25	0.698	0.698	0.081	0.077
	No 75	0.585	0.585	0.000	
Decreased libido	Yes 54	0.289	0.690	0.000	0.141
	No 46	0.250	0.585	0.006	
Menstrual irregularity	Yes 58	0.271	0.705	0.000	0.037*
	No 42	0.173	0.465	0.000	

Table 6: *AMH and the Perimenopausal symptoms, Symptomatic &asymptomatic.*

* = statistically significant.

Discussion

There is no specific test available that can exactly tell the onset of menopausal transition. However FSH, Inhibin and E2 and ultrasound evaluation of antral follicle count have been studied in this regard. Questionnaire based performas have been used to study the symptoms and predict the onset of menopause. This method has also been used by Papini., *et al*. [6] who used the Menopause Symptoms Checklist (MSC) [7] which assesses common physiological symptoms associated with menopause.

The present study is also a questionnaire based study. The menopausal symptoms were grouped under various subgroups and the incidence of each complaint was studied. The symptoms mentioned above are commonly experienced by many women in their late reproductive life and are an indicator of their declining ovarian reserve, if not explained by any other pathology.

A study from Thailand showed many symptoms to be significantly related to the menopause transition status (such as hot flushes, an upset stomach, insomnia, and urinary symptoms) and only night sweats and joint aches and pains were significantly associated with the post-menopausal status [8]. Our study group showed that the maximum number of women complained of complaints listed under the 'Others' group which included Insomnia, mood changes, depression, dry skin and decreased libido. Menstrual irregularities were lowest in all age groups.

We also observed younger Perimenopausal women (35-40 years) were symptomatic for most of the symptoms. A decreased value of AMH suggests that menopause can be expected in the coming few years. McKinlay, Brambilla and Posner [9] have demonstrated similar outcomes suggesting that onset of menopausal transition period (accompanied with onset of undefined symptoms) ultimately lead to menopause. This finding suggests that depletion of ovarian reserve starts much earlier than the average age of menopause.

This fact has been supported by certain other studies which have shown that fixed time intervals exist between the first decline in fertility, the end of fertility and onset of menopause [10-12]. The time interval from the decline in fertility to the menopause has been estimated to be around 20 years [13,14]. Thus, a woman entering menopause at the age of 45 years may already experience an age-related decline in her ovarian reserve at the age of 25 years.

The alteration of endocrine hormones regulating folliculogenesis plays a major role in depletion of primordial follicles (follicular atresia) through apoptotic or other pathways [15-18] followed by permanent cessation of menses (menopause). This early shift in menopausal transition could be due to phenomenon controlled by genetic factors mostly [19-21] along with a few environmental factors such as smoking [22,6], infection and other chemotherapeutic drugs 61/18.

Our study also showed decreased AMH levels in young women in the reproductive age group. Nikolaou and Templeton have reported that the commencement of menopausal symptoms at an earlier age is coupled with early initiation of sub-fertility and transition to menstrual cyclical irregularity is associated with early ovarian ageing [23]. AMH is an upcoming biomarker being widely appreciated for its effectiveness as a novel marker of ovarian reserve [3-5]. The age-related decline in serum-AMH level has been observed in several studies [24-26].

Our study also confirmed this association. It was found that serum AMH levels decreased with the increasing age of a woman in the younger Perimenopausal group from 35-40 years (n = 44) the mean AMH was 0.465 ng/ml, between 41-45 years (n=34) a mean 0 .177ng/ml was detected, the value dropped to0 .032 ng/ml in the age from 46-50 years (n = 15) there was a further fall in its level and the mean was 0.217ng/ml at 50 years (n = 7) and above. This comparison was statistically significant (P value .034). A statistically significant association in mean AMH levels of symptomatic and asymptomatic women was found in relation to dyspareunia, muscular and joint pains.

The results show that serum level of Anitmullerian hormone gradually declined with the increasing age and was lower in symptomatic women, which gives a clue that AMH is indeed a marker of declining ovarian reserve. This has been supported by many other studies also. Annemarie et al24 described in their data that serum level of Anitmullerian hormone in normal ovulatory women decrease over time and decrease with advancing age before other age related changes occur.

The Michigan Bone Health and Metabolism study also made an attempt to find an endocrine biomarker for predicting the final menstrual period by studying hormone assays of AMH, inhibin B, and FSH in 300 follicular phase specimens from 50 women in pre- and

Perimenopausal [27]. The study concluded that out of these hormones, AMH was the best marker; however their assay was not sensitive enough to follow levels sufficiently close to the time of menopause [27].

AMH levels declined to low and non-detectable levels 5 years before the final menstrual period [27]. Baseline AMH level was associated with age at menopause [27]. Similar results have been reported in some of the recent studies too [6] suggesting that AMH blood levels in women declines gradually with age. Although serum FSH levels also increase with diminishing cohort of growing follicles, but it has been observed that FSH is not a good correlative factor in younger aged women (i.e. from 20-35 years) [28]. Shin., *et al*. in 2008 [29] reported that AMH and Inhibin B were undetectable after attaining menopause, but FSH, E2 and LH were found to be stably expressed even after menopause.

Conclusion

Numerous studies support the usefulness of Anitmullerian hormone as a marker of ovarian reserve however there is paucity of literature that defines its role in relation to menopausal symptoms. Till date no Indian study has been published which has studied the effect of comparison between AMH and Perimenopausal symptoms. This is one of the first studies studying this correlation.

AMH was studied with all the symptoms described and we found that woman with a particular symptom had lower AMH compared to the woman who did not have that symptom. In all the age groups maximum women were symptomatic for group taken as others. (Insomnia, mood changes, depression, dry skin and decreased libido).Menstrual irregularities were lowest in all age groups.

The comparative analysis of AMH with hot flashes, night sweats, vaginal dryness, dyspareunia muscular pains, joint pains and menstrual irregularities had statistically significant correlation. Most of the women had more than one symptom. This makes it evident that AMH is good marker of the remaining primordial follicles and their fall with the reproductive ageing makes women symptomatic for Perimenopausal complaints.

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