



## Anti-Müllerian hormone is a predictive biomarker for ovarian reserve in young women with ovarian failure – A preliminary study from Southern India

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### Abstract:

The aim of the present study is to investigate whether the low circulating systemic Anti-Müllerian hormone (AMH) can be a biomarker to diagnose Premature Ovarian Failure (POF) and used as a prognostic tool to predict POF in southern Indian women. A case-control study conducted in 150 subjects by enrolling 75 POF patients and 75 healthy controls from June, 2012 through November, 2014. Hormonal profile of AMH and FSH levels were assayed and correlated with age. Patients and controls were categorised into three age groups Group I (17-24 years), Group II (25-32 years) and Group III (33-39 years). Results revealed that the mean age at onset of the patients was  $26.14 \pm 5.74$  years. Seven percent of the patients were with family history of infertility, in whom 80% showed undetectable AMH levels ( $<0.029$  ng/ml). Significantly decreased AMH levels were recorded in patients than controls ( $0.05 \pm 0.10$  ng/ml, range 0-0.6 ng/ml vs  $2.60 \pm 0.95$  ng/ml, range 0.8-4.2 ng/ml;  $p < 0.001$ ) suggesting the low ovarian reserve in POF women. The AMH levels in both patients and controls were negatively correlated with their age, whereas, a weak positive correlation was observed between FSH and AMH levels ( $p < 0.05$ ). Age stratified data proved that irrespective of age POF women have very low/undetectable AMH levels. Our findings suggest that AMH is a potential biomarker for POF and the patients with family history of infertility are prudent to estimate their AMH levels at an early age for further implementation of pregnancy strategies. To support our results from this preliminary study among south Indian women, further studies are warranted with large sample size to implement in the reproductive medicine.

**Key words:** Age stratification, Anti-Müllerian hormone, Family history of infertility, Ovarian Reserve Premature Ovarian Failure, south India

### Introduction:

Premature ovarian failure (POF) is one of the infertility associated endocrine disorders characterised by the loss of functional ovarian follicles during the reproductive age that affects 0.9-2% of females prior to 40 years and 0.1% of women before 30 years <sup>[1,2]</sup>. Till date, the cause for POF is largely unexplained; however, various rationales based on iatrogenic, infections, autoimmunity, X-chromosome abnormalities and autosomal genetic conditions have been advanced to explain this heterogeneous condition <sup>[3]</sup>. The condition occurs in 10-28% of women with primary amenorrhoea and 4-18% in those with secondary amenorrhoea <sup>[4]</sup>. The disease is idiopathic in 90% of cases but can be familial (4-33%) or sporadic <sup>[5]</sup>. Idiopathic POF represents the most extreme phenotype of diminished ovarian reserve (OR) at younger age. Clinically, POF is recognized as secondary amenorrhoea lasting for at least 4-6 months with Follicle stimulating hormone (FSH) levels exceeding 40 IU/l before 40 years. It is a complicated multifactorial disorder that hampers the fertility potential of younger women years before normal menopause and generally characterised by decreased Anti-Müllerian hormone (AMH) secretion <sup>[6]</sup>. Infertility, the most distressing consequence of POF is associated with diminished OR, and women with family history of infertility might be at risk in the predisposition of this clinical condition at an early age. Prediction of OR has long been the best tool through various endocrine [FSH, AMH, inhibin-B, estradiol etc] and ultrasound [ovarian volume, antral follicle counts (AFCs)] tests to clinically assess fertility potential, guide infertility treatment and also to estimate time to the final menstrual period <sup>[7]</sup>. AMH is a paracrine modulator of follicular development and dominant follicle selection produced by ovarian granulosa cells <sup>[8]</sup>. The hormone originally known to inhibit the development of Mullerian ducts in male embryos and also proved to be an excellent marker in assessing OR in women <sup>[9]</sup>.

The AMH, also known as Mullerian Inhibiting Substance (MIS) is a glycoprotein and a member of the transforming growth factor- $\beta$  family produced by granulosa cells of preantral and small antral follicles. Serum AMH levels rise in the weeks following birth and reach its highest level after puberty<sup>[10]</sup>. Studies reported that AMH is expressed only in healthy follicles; whereas, the levels decline with increasing age in women over 25 years, disappear at menopause, and correlate with the number of remaining primordial follicles<sup>[11-13]</sup>. In addition to the support that AMH gained from several studies as a good predictor of OR, the circulating AMH serum levels exhibit less intra and intercycle fluctuations<sup>[14]</sup>.

It has been stated in the literature that the mean of AMH levels differ among races and ethnicities<sup>[15]</sup>. The cut-off values of AMH levels found in various studies differ between 0.1 and 2.97 ng/ml (0.7 and 20 pmol/l) for low response to ovarian hyperstimulation<sup>[16]</sup>. Hence, improving our understanding of racial differences in ovarian reserve and their underlying causes may be essential for timely interventions which may prolong reproductive lifespan in women. The follicle populations as well as the serum AMH levels change with advancing age, therefore we expect AMH may differently reflect the OR at different age groups. The present study focuses to estimate and analyse age related AMH levels among POF patients and controls. The significance in estimating the AMH levels at different age groups may provide vital information in the fertility treatment strategies. There have been various national and international studies that reported low serum AMH levels in sub-fertile women<sup>[17]</sup>, PCOS women<sup>[18]</sup>, POF women<sup>[19]</sup> and women carrying Fragile X mental retardation gene 1 (FMR1) CGG permutation allele<sup>[20]</sup>, compared to normal menstruating females. However, AMH based reports in relation to POF conditions are not to be found among south Indian women.

In the light of literature support, for the first time from southern India we conducted a case-control study to investigate whether AMH could be considered as a prognostic biomarker of functional OR in POF patients. Further, we hypothesise that family history of infertility may be considered as one of the characteristic features to predict POF in the family. Our results may help the clinicians to provide necessary management to south Indian POF patients in the reproductive treatment.

## **Material and Methods:**

### ***Sample recruitment:***

This is a case-control study conducted during the period June, 2012 through November, 2014, with the approval of institutional ethical committee, Osmania University, Hyderabad, India. After a written informed consent, patients and controls were sampled in collaboration with reproductive, endocrine and infertility clinics of Hyderabad, Telangana State, India. The inclusion criteria of 75 diagnostically proved idiopathic POF patients was, women aged between 17-39 years with normal karyotype and women reached secondary amenorrhoea. Patients receiving hormone therapy, radiotherapy/chemotherapy, with a history of ovarian surgery or family history of POF, with autoimmune diseases or infertility related complications were excluded from the study. A group of 75 healthy females with regular menstrual cycles, having no fertility related complication and family history of POF, and were similar to patients age range were recruited as control group.

Detailed information of age, age at onset of the disease, age at menarche, age at marriage, body mass index (BMI), menstrual and obstetric history, if any autoimmune or genetic disease in the participated subjects or in the family along with three generation pedigree were taken from all the study participants. For the additional analysis, we also included the fertility status of the family members, with no POF in case of females. Information on family history of infertility was exploited with different clinical and hormonal parameters. We intend to highlight the importance of implementing the necessity to estimate the AMH levels of other female family members when there is already a family member with infertility, to predict POF risk. Patients and controls were assessed in three groups: Group I (17-24 years) Group II (25-32 years) and Group III (33-39 years) for age-stratified analysis.

### ***Hormonal assays:***

Five millilitres of peripheral blood was collected from all the patients on random days. On the other hand, in order to estimate the FSH levels, controls provided the blood sample during the early follicular phase (Day 3) of the menstrual cycle. Following centrifugation, serum was separated from the blood and stored at  $-20^{\circ}\text{C}$  until respective assays were performed in sets.

The AMH levels were estimated using an Enzyme-linked Immunosorbant Assay (ELISA, Ultrasensitive AMH/MIS, Ansh Labs, USA), which is a quantitative three-step sandwich type immunoassay.

The lowest amount of AMH/MIS in a sample that can be detected with a 95% probability is 0.029ng/ml, and the levels below this limit were considered as 'zero' for our statistical analysis. Furthermore, FSH levels were also measured by using a commercially prepared kit produced by Calbiotech (CA, USA), a solid phase direct sandwich ELISA method.

**Statistical analysis:**

Descriptive statistics was executed for selected variables; we represented the continuous data as mean ± standard deviation (SD) and categorical data as percentages. Mann-Whitney U-test and Student t-test was used to test the mean difference between two groups, and one-way analysis of variance to test the difference between the three age groups. Pearson

correlation coefficient was used as appropriate. Significance was defined at  $p < 0.05$ . All statistical analyses were performed using statistical software, SPSS version 19, IBM, USA.

**Results and Discussion**

General and clinical characteristics of patients and normal healthy controls are given in the table . The mean age of controls and patients at the time of recruitment was  $27.16 \pm 6.80$  and  $28.17 \pm 5.21$  years respectively ( $p > 0.05$ ). The mean values of age at menarche, BMI, serum FSH, and AMH levels differed significantly between patients and controls in ( $p < 0.05$ ). All the patients exhibited secondary amenorrhoea with a mean age at onset of  $26.14 \pm 5.74$  years. Of the total patients, 68% had history of irregular menstrual cycles before disease onset, and 71% were with primary infertility. Consanguineous marriages were reported in one control and three patients.

**Table1: Distribution of Clinical, epidemiological characteristics and hormonal levels of POF Patients and controls**

	Controls (75) Mean±SD	Patients (75) Mean±SD	t-value
Age at recruitment (yrs)	27.16±6.80	28.17±5.21	1.02 <sup>NS</sup>
Age at onset (yrs)	-	26.14±5.74	
Age at menarche (yrs)	14.16±1.05	13.20±1.26	5.07 <sup>a</sup>
Age at marriage (yrs)	23.04±2.23	23.21±4.86	0.25 <sup>NS</sup>
BMI (Kg/m <sup>2</sup> )	22.17±3.34	24.63±2.41	5.17 <sup>a</sup>
FSH levels (mIU/ml)	10.02±5.10	68.03±18.51	26.16 <sup>a</sup>
AMH levels (ng/ml)	2.6±0.95	0.05±0.11	10.57 <sup>a*</sup>
<b>Secondary amenorrhoea diagnosis</b>		<b>n=75 [%]</b>	
Primary Infertility		53 [71]	
Secondary Infertility (at least conceived once)		14 [19]	
Spontaneous miscarriages		4 [5]	
Unmarried subjects		4 [5]	

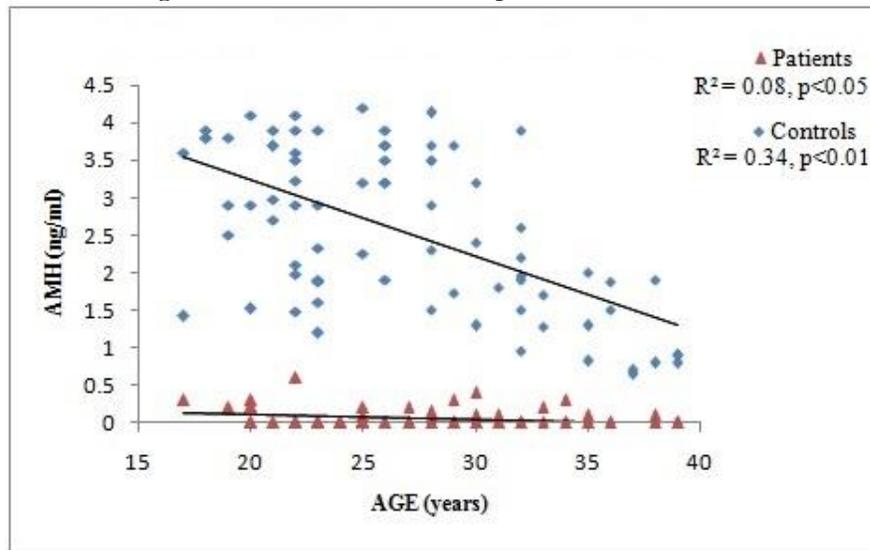
NOTE: NS- Not significant; <sup>a</sup> $p < 0.05$ ; <sup>b</sup> $p < 0.001$ ; \*Mann-Whitney U test (Z-value)

**AMH profile:**

Age related decline in AMH levels were seen significantly in controls (figure1). As described by Pingy et al <sup>[21]</sup>, the circulating AMH levels found in controls were mostly within the normal range (2.2 – 6.8ng/ml) with a significant decline after 32 yrs.

When comparing AMH levels between the groups, patient group exhibited significantly low mean levels ( $0.05 \pm 0.11$ , range 0-0.6ng/ml) as in normal post-menopausal women<sup>[19,22]</sup> than the control group ( $2.6 \pm 0.95$ , range 0.8-4.2ng/ml) (Man-Whitney U-test,  $p < 0.05$ ).

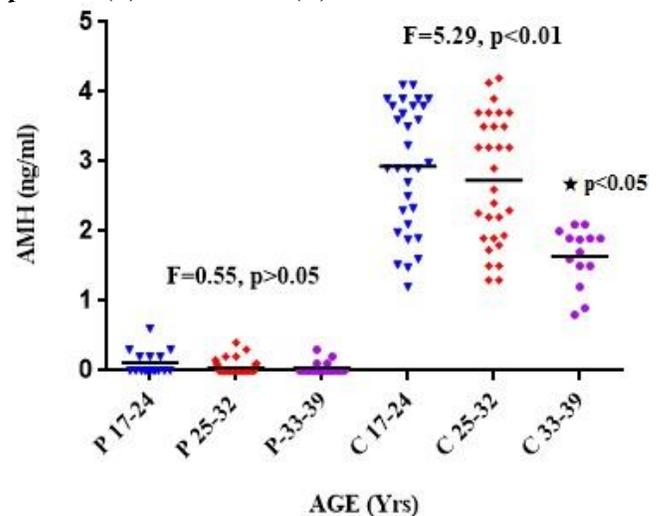
Figure 1: Serum AMH levels in patients and controls



Ovarian biopsy studies revealed that POF is associated with the absence of or a very low number of ovarian follicles [19, 23]. In our study, serum AMH levels were undetectable in 75% of patients which is similar to women with bilateral oophorectomy [22] suggesting the reduced ovarian reserve. The detectable minimum and maximum AMH values were recorded as 0.08ng/ml and 0.6ng/ml respectively. Though detectable in 19 patients with a mean of  $0.20 \pm 0.12$ ng/ml, one patient showed AMH levels below the menopausal threshold, i.e., 0.086ng/ml [24], 16 reported  $< 0.4$ ng/ml, which is considered as extremely low [25], and two patients who were below 30 years had 0.4 and 0.6ng/ml. Further, a negative correlation was observed between age and serum AMH levels both in patients ( $r = -0.28, p < 0.05$ ) and controls ( $-0.63, p < 0.001$ ), whereas, a weak positive correlation between FSH and AMH was noticed ( $r = 0.21, p < 0.05$ ).

It is known that AMH levels are used in reproductive medicine to predict ovarian response in controlled ovarian stimulation environment [26]. Though 5-10% of POF patients have a better chance to conceive [27], a poor response to in-vitro fertilization (IVF) was correlated with decreased AMH levels [28]. Our results with the support of Wang et al [29] suggest that patients with significantly low AMH levels may contain inadequate number of follicles, hence, might have low possibility of achieving a successful pregnancy through IVF. However, treatment for infertility is not an option for POF patients with the exception of oocyte donation [30].

Figure 2: AMH levels in Age stratified data of patients (P) and controls (C)



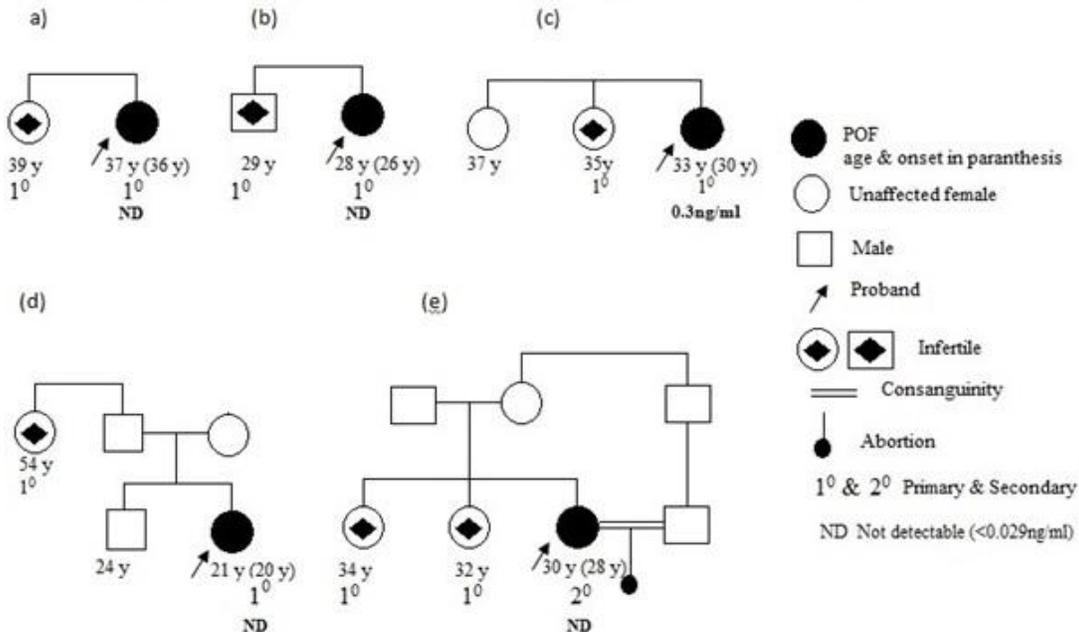
The age stratified data showed that the AMH levels decreased slightly in patients and significantly in controls with advancing age (Fig 2). The mean AMH between patients and controls was significantly differed in all the three age groups ( $p < 0.05$ ). AMH levels in control subjects significantly varied between the three age groups ( $F = 5.29, p < 0.001$ ), with a significant decline in Group III. On the other hand, patients showed no significance difference between the three groups suggesting that AMH is an independent biomarker of age, a clinical hallmark of POF condition ( $F = 0.55, p > 0.05$ ). However, the mean AMH levels in Group 1 patients ( $0.11 \pm 0.18$ ng/ml) were more than the menopausal threshold [24] but within the cut-off values for poor ovarian response [31]. These significant results indicate interaction between ovarian heterogeneity and age of the patients

was genetically predetermined at extreme age groups [32]. Our results suggest that the chance of conception through IVF is not negligible in the younger patients even with the prediction of a very low OR [25].

Another significant aspect we found in our study was that nearly 7% (5/75) POF women have family

history of infertility, and none in the control group. This information led us to ascertain intriguing summary of certain clinical features (Fig 3). The pedigrees showed that AMH was undetectable in 80% patients; moreover, four of five patients reported with primary infertility.

Figure 3: Pedigrees showing family history of infertility in POF patients



Interestingly, in two families infertility is seen in brother (Fig 3b) and paternal aunt (Fig 3d), which heaved the support of X-chromosome involvement (FMR1 gene) in POF pathogenesis. Several studies including a recent review reported the association between AMH and infertility as well as AMH and familial POF [31]; however, there are no studies on AMH in relation to POF condition with family history of infertility. To our knowledge, this is the first study addressing the role of AMH and family history of infertility in south Indian women with POF. However, the results from this preliminary study should be continued with a larger sample size.

**Conclusion**

For the first time from south India the present study deals with the assessment of AMH levels in POF women and healthy controls. The outcome of our preliminary study with limited samples, certainly suggested that AMH could be considered as a better future marker by the clinicians to assess OR in the fertility treatment options. Comparatively, patients showed significant high FSH and low AMH levels

than controls. The age stratified data with varying levels of AMH in younger and older group of patients, could be explained by the ovarian heterogeneity at extreme age groups that might be genetically predetermined. AMH is a marker for predicting the earliest signs of POF rather than the onset of infertility among women with family history of infertility. To address the epidemic of infertility, if a woman is offered an indication of her OR at an early stage through AMH estimation, pregnancy may still be viable to the patient perhaps with suitable assisted reproductive technique. Based on our findings, we also propose that future studies should involve various familial factors including familial infertility in causing the POF condition on par with hormonal estimation to recognize persistent follicles and to decide on quick management strategies. However, the results showed and the suggestions made in our study should be viewed with caution and replicated in large samples before implementing clinically. Results obtained from control data can be exploited by future studies that involve AMH levels to predict the OR in south Indian women which facilitate reproductive life planning.

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