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Early Follicular Anti-Mullerian Hormone and Antral Follicle Count as an Indicator of Ovarian Reserve: A First Pilot Study in the Nepalese Population.

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ABSTRACT

Background: Anti-Mullerian Hormone (AMH) is produced by the granulosa cells of primary, preantral and greater antral follicles, and plays an imperative role in human folliculogenesis. It is a measure of ovarian function, useful in assessing conditions such as polycystic ovary syndrome and premature ovarian failure. The objective of this study was to evaluate the correlation between chronological age with levels of antral follicle count (AFC), and AMH. **Methods:** Anti-Mullerian Hormone (AMH) is produced by the granulosa cells of primary, preantral and greater antral follicles, and plays an imperative role in human folliculogenesis. It is a measure of ovarian function, useful in assessing conditions such as polycystic ovary syndrome and premature ovarian failure. The objective of this study was to evaluate the correlation between chronological age with levels of antral follicle count (AFC), and AMH. **Results:** The correlations between AFC, AMH, and age were statistically significant. The lowest value of AMH and AFC were 0.09 ng/ml and 1 respectively. The highest values of AMH and AFC were 15.90 ng/ml and 14 respectively. The study revealed consistent levels of AMH up to 25 years of age, whereas decline in the levels post 25 years. **Conclusion:** AFC is being used as a marker of ovarian reserve; AMH levels have a positive correlation with AFC and can be measured on any day of the cycle. Anti-Mullerian hormone is believed to be of paramount importance in its role as an endocrine marker for assessment of decline of the ovarian pool in healthy women; thus, it has a potential ability to predict future reproductive lifespan.

Keywords: Anti Mullerian Hormone, Follicle Stimulating Hormone, Antral Follicular Count, Fertility, Ovarian reserve.

INTRODUCTION

Anti-Mullerian hormone also known as AMH is a protein that is encoded by the AMH gene (in humans), named after Johannes Peter Muller.[1] It is mainly secreted by the granulosa cells of ovarian early developing follicles, structurally related to inhibin and activin and is a member of the transforming growth factor-β (TGF-β) family.[2,3] It has been mainly studied for its regulatory role in male sex differentiation, produced by the Sertoli cells of the fetal testis, induces the regression of the Mullerian ducts, the anlagen of the female reproductive tract.[4] In the past, AMH was known mainly for its role in the differentiation of male sexual characteristics.[5]

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Expression of this hormone in women is detected at 36th week of gestation, rises during puberty and declines from 25 years of age to imperceptible levels at menopause.[6] AMH is produced by the granulosa cells of primary, preantral and greater antral follicles, suggesting its imperative role in human folliculogenesis.[7]AMH is a measure of ovarian function, useful in assessing conditions such as polycystic ovary syndrome and premature ovarian failure.[8]Abnormalities in the level of this hormone may indicate a woman's diminished ability or inability of conception.[9,10] AMH levels have a positive correlation with basal antral follicle count (AFC) measured by ultrasonography and can be measured on any day of the cycle.[11]

The term "ovarian reserve" refers to the quantity and quality of a woman's current reservoir of oocytes and is closely associated with reproductive potential.[12] Antral Follicular Count refers to the total number of resting follicles in both ovaries at the beginning of proliferative phase of the menstrual cycle. They are approximately 2-6 mm in size and a count of 8–10 is considered as a normal range. They are being used as a marker of ovarian reserve.[13] Measurement of

ovarian reserve is very important in predicting a woman's response to various fertility treatments and helps us decide on appropriate fertility medication dosage levels for that treatment.[14]Anti-Mullerian hormone is believed to be of paramount importance in its role as an endocrine marker for assessment of decline of the ovarian pool in healthy women; thus, it has a potential ability to predict future reproductive lifespan.[15]

Various parameters used for evaluation of ovarian reserve include woman's age; assays of serum Follicular Stimulating Hormone (FSH) in the early follicular phase, inhibin B, E2, basal estradiol with varying degrees of reliability. La Marca *et al.* revealed that serum AMH levels do not change throughout the menstrual cycle, thereby, allowing random timing of AMH measurement during the menstrual cycle. Hence, it has been suggested that serum AMH values are more convenient and more effective than other serum ovarian reserve tests.[16]

Hence, the present study was undertaken to evaluate the correlation between chronological age with AFC, and AMH and predicting the fertility pattern in females. To the best of our knowledge, the present study is the first of its kind conducted in Nepalese population.

MATERIALS AND METHODS

The study was conducted on 100 women of child bearing age attending OPD and IPD of Nepalgunj Medical College Teaching Hospital, Banke, Nepalgunj from the period of January 2015 to February 2016. Approval by ethical committee and signed consent was obtained from all the patients enrolled in the study. Patients with regular menstrual cycles (21-35 days), adequate visualization of both ovaries without evidence of abnormality, were included in the study. However, women presenting with polycystic ovary syndrome, endometriosis, autoimmune disorders, history of hormonal therapy, lactation, history of ovarian surgery, drugs affecting ovarian function were excluded from the study.

Serum AMH levels were assessed on any day of the menstrual cycle by using a second generation enzyme immunoassay (AMH Gen II ELISA, Beckman/Coulter, USA). The minimal detection limit for the AMH assay was 0.017 ng/ml. AFC (follicular measuring 2 to 9 mm in diameter) i.e. the total number of antral follicles were counted bilaterally by transvaginal ultrasonography using a VOLUSON S6 (General Electric, USA, 2011) on the 3rd day of cycle, with a 4-10 MHZ multi-frequency ultrasound probe.

Data was analysed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL) using Chi-Square test and *t* test. P <0.05 was considered as statistically significant.

RESULTS

All patients in our study belonged to the age group of 18–45 years. Among the study group, 49 patients (49%) were between the age of 18-25 years, 42 patients (42%) were between 26 and 35 years, and 09 patients (09%) were between >35 years old. [Table 1]

[Table 2] depicts the minimum and maximum range of AMH and AFC. The normality of data distribution was confirmed through *t* test. [Table 2 and 3]

The correlations between AFC and AMH and age were statistically significant. The lowest value of AMH and AFC were 0.09 ng/ml and 1 respectively. The highest values of AMH and AFC were 15.90 ng/ml and 14 respectively. The peak age when AMH were maximum and minimum was 22 and 29 years and respectively. The peak age when AFC were maximum and minimum was 24 and 31 years and respectively.

Table 1: Age range of patients.

Age range (in years)	AMH and AFC (in %age)
18-25	49
26-35	42
>35 years	09

Table 2: Minimum and maximum range of AMH and AFC.

Range (in ng/ml)	AMH	AFC
Minimum	0.09	1
Maximum	15.90	14

Table 3: Normality tests for AMH with age.

		Age	AMH
N		**100**	**100**
Normal parameter	Mean	28.29	4.42
	Std. Deviation	6.26	3.41
Extreme variance		39.25	11.60
t-value		33.13	
Asymp. Sig. (2-tailed)		< .00001	

Table 4: Normality tests for AFC with age.

		Age	AFC
N		**100**	**100**
Normal parameter	Mean	28.29	6.30
	Std. Deviation	6.26	6.12
Extreme variance		39.25	37.49
t-value		31.67	
Asymp. Sig. (2-tailed)		< .00001	

DISCUSSION

Serum AMH is a new method used for determination of the ovarian reserve, giving a more accurate measurement. AMH is produced by the granulosa cells of follicles, accurately measures the active follicle pool, as active follicles only produce AMH. Unlike other biochemical markers, it does not

exhibit inter-cycle variability and can be measured on any day of the cycle. AFC is considered to have the best discriminating potential for a poor ovarian response compared to the total ovarian volume and basal serum levels of FSH, E2, and inhibin B on day 3 of the cycle.[17]

Kalaiselvi *et al*. conducted a study to assess the ovarian reserve in women of the fertile and sub-fertile groups with regular cycles, by estimating the level of AMH and hormones like FSH and E2 and calculating the ovarian volume and AFC. It was found the AFC and the ovarian volume were negatively correlated with the age, whereas, the ovarian volume was positively correlated with the AFC and FSH negatively correlated with the AFC. They concluded that AMH can be considered as a marker for assessing the ovarian reserve, as it is cycle independent as compared to the other hormones.[18]

Jyoti *et al*. conducted a study to assess the relationship between obesity and AMH as a serum marker of ovarian reserve in premenopausal women. The study sample was divided into 2 groups: 50 participants (non-obese) with a BMI < 30 kg/m^2, and 50 participants (obese) with a BMI of 30 to 35 kg/m^2. The AMH level in-group A was significantly lower as compared to group B. In conclusion, there was significant difference in serum levels of AMH between obese and non-obese women indicating that obesity is likely to affect ovarian reserve in the premenopausal age group.[14]

In the our study, AMH and AFC levels started declining with age. These results were consistent with the study conducted by Faddy *et al.* who stated that menopause is triggered by the number of ovarian follicles falling below a threshold number and is irreversible because oogonial stem cells disappear after birth.[19] Another study conducted by Jyoti *et al*. concluded that there was a significant decrease in serum AMH level with increase in BMI in late premenopausal women.[14] A study conducted by Mitchell *et al*. concluded that AFC decline with age in a Caucasian population is best described as a gradual acceleration in decline with age.[20]

The current study revealed consistent levels of AMH up to 22 years of age, whereas decline in the levels post 25 years. These findings were in concordance with the study conducted by Fong *et al*. where they concluded that AMH levels increase during infancy, whereas a plateau is observed from adolescence until the age of 25 years. From the 25 years, serum AMH levels correlate inversely with age, implying that AMH is applicable as a marker of ovarian reserve only in women of 25 year old and older.[21]

CONCLUSION

In conclusion, the findings in our study revealed that AFC and serum AMH decreases with age. AFC is being used as a marker of ovarian reserve; AMH levels have a positive correlation with AFC and can be measured on any day of the cycle. Anti-Mullerian hormone is believed to be of paramount importance in its role as an endocrine marker for assessment of decline of the ovarian pool in healthy women; thus, it has a potential ability to predict future reproductive lifespan.

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